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CONTENTS

The relation of the cabbage maggot and other insects to the spread and development of club root of Cruciferae ..... Della E. Simmons 857

Host specialization of barley leaf rust, Puccinia anomala. ..... E. B. Mains 873

Studies on the properties of the bean-mosaic virus ..... T. C. Parker 883

Report of the Cotton-Root-Rot Conference at Temple, Texas. ..... Wilmon N. Edwards and D. C. Neal 897

Attempts to induce "sporeforming" in Fusarium moniliforme. ..... Leon H. Leukel 903

To what extent is "spore formation" in apple fruit caused by the freezing of the flowers? ..... Geo. L. H. McDonald and A. J. Hawkins 907

Wheat take-all symptoms compared with injuries caused by chinch bugs. ..... Homer Shapovalov 909

A washing device for isolation work with plant material. ..... J. M. Armstrong 911

Physiologic resistance in cotton ..... R. H. Hoover, H. K. Chen, and T. F. Yu 913

Phytopathological Notes 917

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PHYTOPATHOLOGY

VOLUME 20 NUMBER 11

NOVEMBER, 1930

THE RELATION OF THE CABBAGE MAGGOT AND OTHER INSECTS TO THE SPREAD AND DEVELOPMENT OF SOFT ROT OF CRUCIFERAE[1]

DELIA E. JOHNSON[2]

INTRODUCTION

In 1927 Leach (11) showed that the seed-corn maggot (*Hylemyia cilicrura* Rond.) is a common agent of dissemination and inoculation of the potato blackleg pathogene. In that paper he briefly called attention to a similar relationship between the cabbage maggot and the soft rot of cabbage. This paper is an account of a study of the bacterial soft rot of cabbage with special reference to the rôle of the cabbage maggot (*Hylemyia brassicae* Bouché) in the dissemination of the pathogene and the development of the disease. Since the completion of this work Bonde (2), in a preliminary note, also has reported the cabbage maggot as a disseminating agent of bacterial soft rot of Cruciferae.

THE SOFT ROT OF CABBAGE

Jones (10), in 1900, proved that the soft rot of cabbage and of many other vegetables is caused by a bacterium which he named *Bacillus carotovorus*. Harding and Morse (9) later showed that the disease can be caused by a group of closely related bacteria, considered by them to be variant strains of the single species, *B. carotovorus* Jones.

Although Jones (10) proved that *B. carotovorus* was a wound parasite and that it could penetrate the unbroken epidermis, the manner in which it is disseminated and inoculated into plants never has been explained satisfactorily. Insects have frequently been mentioned as agents of dissemination. Chupp (5) in his general discussion of soft rot of vegetables makes the following statement: "The organism, *B. carotovorus,* is common on so many hosts both in storage and in the field, that it lacks no opportunity of

[1] Published with the approval of the Director as Paper No. 938 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to express her appreciation to Dr. J. G. Leach, under whose direction the work was carried on, to Dr. E. C. Stakman for valuable assistance, and to Dr. R. U. Cotter for specimens of the onion maggot. She is indebted also to Dr. C. E. Skinner of the Department of Bacteriology for criticizing the manuscript.

becoming established in new crops in summer. Carried by insects or in any other manner in which microscopic bodies may be disseminated, the bacteria gain an entrance into the injured tissue, where they soon dissolve the middle lamella, leaving only a slimy mass of free cells;" also, "insects and cultivating tools carry the organism in the field." In 1926 Leach (12) showed that the dipterous insects, *Elachiptera costata* Leow. and *Scaptomyza graminum* Fall., are capable of disseminating the pathogene and of inoculating it into celery, where it produces a heart rot.

Symptoms.—Soft rot of cabbage may become manifest in several ways. In the field it is most destructive as the so-called "stump rot." Stump rot appears most frequently rather late in the season when the plants approach maturity. The first symptoms usually consist of a wilting of a few of the outer leaves and a cessation of growth. Frequently the head may appear normal when viewed casually. However, upon careful examination, it usually will be found that the center of the head is a mass of decaying tissue, extending downward into the pith of the stem. In advanced stages such heads may be pulled from the stem easily. Observations throughout the season show that the decay arises first in or on the base of the older leaves, whence it spreads upward into the center of the head. After the decay has reached the head, it is only a matter of time until the entire head is reduced to a mass of rotting tissue, leaving the stem as a ragged stump with the core completely destroyed.

Soft rot is primarily a disease of the succulent tissues of the plant; the woody portions are not affected. On young plants it is commonly confined to the tissues close to the injuries caused by the cabbage maggot, as will be pointed out later. Soft rot is very destructive to cabbage heads in storage or in transit, especially if the disease has been common in the field, and if the cabbage is stored in poorly ventilated places at relatively high temperature.

THE CABBAGE MAGGOT

The cabbage maggot (*Hylemyia brassicae*) is a common and well-known insect. It has been described so adequately by Schoene (15), Gibson and Treherne (6), and other entomologists that only a brief description of it will be given here. It is distributed throughout the United States, Canada, and Europe. It attacks all members of the Cruciferae, being especially destructive to cabbage, cauliflower, and radish. The first brood of the adult fly emerges in the spring and early summer. It resembles the house fly, but is smaller, being only about one-fourth of an inch long, with a narrower body and proportionately larger wings. It is grayish, with three dark stripes on the thorax and one along the abdomen, and has many stiff hairs on the body. The male is slightly smaller than the female. Morpho-

logically there is no difference between the adult female fly of the cabbage maggot and that of the seed-corn maggot, but the male adult fly of the cabbage maggot can be distinguished from that of the seed-corn maggot by the presence of a tuft of bristles on the base of the hind femur.

The first brood of flies deposits eggs near the base of the stem of the young host plants. The eggs hatch after a few days. The maggot is white or yellowish and tapers slightly toward the head. When full-grown it is about one-third of an inch long. It has a pair of strong, dark colored, hook-like, rasping, downward-projecting jaws. The newly hatched maggot begins at once to rasp the surface of the young roots, gradually mining into them. It finally ceases feeding, and the skin hardens, forming the puparium, within which the larva transforms to a pupa. Later the adult fly emerges from the puparium.

The attacks of the maggot kill or severely injure the young plants. Although soft rot is frequently present on such plants, it is usually obscured by the mechanical injury caused by the larvae. Because of the relatively large proportion of lignified tissue in the stems of young plants, the rot seldom spreads far beyond the tissues injured by the maggots.

The later broods often deposit eggs at the base of the leaves of the older plants. When these hatch, the larvae burrow into the leaf base and often into the head of the cabbage plant. These tissues are very succulent and especially susceptible to soft rot; following such maggot injury, they frequently decay, giving rise to the condition known as stump rot. In the fall the maggots and the puparia are often found wedged between the leaves of the head, the flies of the last brood frequently laying the eggs on or between the leaves of the well-developed heads. The insect overwinters in the puparial stage in the soil or in the refuse from cruciferous plants. In mild climate it may also overwinter in the adult stage.

Entomologists have observed for many years that laceration of the cabbage-plant tissue by the cabbage maggot often is followed by decay. According to Slingerland (16), Ormerud stated in 1892 that "the maggots bore into the roots and cause mischief, sometimes to a serious extent by the decay so originated." Slingerland expresses entire agreement with this view. Schoene (15) says that "the lacerations of the roots of the cabbage and cauliflower are often accompanied by a decay of the injured part," and "the newly made tunnels are clear, but later they become brown and discolored due to the decay of the exposed tissues."

THE PRESENCE OF THE SOFT-ROT BACTERIA IN THE VARIOUS STAGES OF THE INSECT

The Puparium.—The first collection of puparia for the study presented in this paper was made in August, 1927, from the second brood of maggots, the puparia being collected from soil around both cabbage and radish

plants. The methods used in the isolation of bacteria from the puparia were, in general, similar to those used by Bacot (1) and by Leach (11).

Young puparia, recognizable by the light brown color, were selected for immediate work, the darker ones, evidently a more advanced stage of the insect, being reserved for the study of the adult fly. The puparia were washed carefully to remove all adhering particles of dirt. Next they were immersed for 5 minutes in 5 per cent alcohol. They were then transferred with a flamed needle to a solution of mercuric chloride (1–1000), where they were kept 3 minutes, after which they were removed aseptically to 50 per cent alcohol and kept there for about a minute, being finally placed in tubes of sterile broth to wash off all trace of the disinfectant. Later they were transferred to slants of sterile agar and allowed to remain for several hours. These slants were kept under observation for bacterial growth, since any growth here would indicate the survival of external contamination of the puparia. The puparia were again washed in sterile broth and transferred to fresh agar slants. Both the broth and the agar slants were saved for further observation for bacterial growth.

Seventy-two of 214 puparia so treated produced no growth in the broth or on the agar slants and were kept to await the emergence of the adult fly. Many of the remainder produced no contamination in the broth, but eventually bacterial growth was observed upon the agar slants. This might indicate that the puparia had been sterile externally but that bacteria had seeped out through the air pores. This growth often produced a soft rot when inoculated into cabbage leaves, but no conclusions were drawn from such inoculations.

Various methods of sterilizing the surface of cabbage leaves were tried. Five per cent Formalin gave good results with potatoes and carrots but was not successful with cabbage leaves. Immersing the leaves in alcohol, then burning off the alcohol, was tried. But this often produced a softening of the tissues. Lugol's iodine solution left fumes which apparently interfered with the development of the inoculum. Mercuric chloride solution (1–1000) was fairly efficient, but the best results were obtained with Semesan and other organic mercury compounds in the usual concentrations. The disinfectants were washed off with sterile water, then the leaf pieces were transferred aseptically to sterilized petri dishes containing several layers of moist filter paper. To accomplish sterilization of the dishes it was necessary to subject them to steam at 15 pounds pressure for at least 30 minutes. Cabbage-leaf sections, so prepared, were incubated at 22° to 25° C. for 48 hours and all discarded which showed any softening of the tissue. At least 4 plates were used as checks. If any softening of the tissue took place in any of the checks, the experiment was repeated.

Various methods of preparing the inoculum were tried. Water suspensions of the organism were found more satisfactory than broth cultures, since saprophytes were not always destroyed, even with the most rigid surface sterilization of the cabbage leaves. Such saprophytes develop readily in broth, and, if a softening of the tissues should occur as a result of high temperature and humidity, erroneous conclusions might easily follow.

From day to day, according to the age of the puparia, adult flies emerged from the puparia previously planted on the sterile agar slants until, in all, 53 flies had been so obtained. Many dragged their wings upon the agar and also left foot tracks. After about 24 hours these marks were clearly defined by bacterial growth. The growth from each of these slants was plated on nutrient agar. Cultures corresponding macroscopically, microscopically, and physiologically to *B. carotovorus* were obtained from 27 of the puparia. These were later found to be pathogenic to cabbage, as will be discussed later. Since many of the cultures so isolated were found to consist of saprophytes, it is evident that colonies of *B. carotovorus* are not readily distinguished from many other bacteria present in the puparia. In order to determine if the soft-rot bacteria might be present on the plates and be missed in the selection of colonies, the remainder of the growth on each plate was washed off with sterile tap water and this bacterial suspension inoculated into cabbage-leaf sections. Washings from 11 plates from which no colonies of *B. carotovorus* had been picked produced soft rot. Thus from 53 flies that had emerged from the puparia upon the agar slants, 38 harbored the soft-rot bacteria in the puparial stage.

A slightly different method for sterilizing the surface of puparia was tried by immersing 19 of them in 95 per cent alcohol, then burning this off. These puparia were placed on agar slants and incubated for 48 hours. Six showed contamination and were discarded. The remaining 13 were tested for surface contamination by placing them in broth and later transferring the sterile ones to agar slants. Seven of them showed no growth, either in broth or on agar, and were crushed in broth with a sterile glass rod. Bacterial growth developed from all of them, four yielding *B. carotovorus*.

Another method of isolation consisted in the removal of the contents of the surface-sterilized and tested puparia by a fine-drawn sterilized pipette. Seven puparia picked from rotting cabbage heads in the field were used for this purpose. Five were successfully surface-sterilized. To insure further against contamination a red-hot scalpel was applied to the parts of the puparia in which the sterile pipette was to be inserted. The contents of the puparia were drawn into the pipette and plated on nutrient agar. The bacterial count on all plates was extremely high, and colonies of *B. carotovorus* were numerous on all the plates, corroborating the writer's anticipation that larvae feeding on decaying plants probably ingest large numbers of bacteria, many of them persisting in viable form in the puparia.

On the other hand, there is some evidence to show that maggots developing on the less extensively decayed tissues ingest bacteria in smaller numbers. One hundred maggots which had been gathered from "tunneled" radishes showing little or no decay were allowed to develop on radishes. The puparia thus obtained were surface-sterilized and tested, 41 being found surface-sterile. Eleven of them were crushed in broth and plated, but colonies of *B. carotovorus* were picked from only one. Since it was possible that the pathogene might have been missed in the selection of the colonies, washings were made from the plates and these suspensions inoculated into cabbage-leaf sections. Only one other plate was found to contain the soft-rot organism. The 30 puparia remaining were surface-sterilized, 23 being found surface-sterile. These were crushed in tap water and inoculations made into cabbage leaves; only 2 produced a soft rot. Bacterial counts were low, ranging from 120 to 800. These results suggested that the bacterial content of the insect probably varies according to the bacterial content of the food ingested and that feeding experiments might show definitely the possibility of such a relationship. The results of such experiments are discussed later.

THE IMAGO

Transmission of viable bacteria to the adult fly.—Four adults emerged from the puparia on agar slants while under the observation of the writer. The interior of the empty puparia was swabbed with a tiny cotton swab and inoculations made from this into sterile broth. When this broth was plated on agar bacteria developed on all the plates and the soft-rot pathogene was obtained from two of them. The bacteria apparently had been voided in viable form in the meconium. This was to be expected, as, in the first experiments conducted on the puparia, the exterior of the emerging flies was evidently contaminated with bacteria, for the tracks from the feet and dragging wings of the emerging fly were outlined after a time by bacterial growth. Undoubtedly, the adult fly always is contaminated with bacteria voided during the puparial stage.

The presence of viable bacteria in the meconium suggested that viable organisms might be harbored also in the gastric tract of the adult. To ascertain if this were true 5 male and 13 female flies, collected from rotting cabbage heads in the field, were surface-sterilized by immersion in alcohol, which was then burned off as an additional precaution. The under side of the abdomen was seared with a red-hot scalpel. The digestive tract was dissected out at this point, crushed in broth, and plated on agar. Colonies of *B. carotovorus* were found in 3 of the male flies and 6 of the females. From one of the male flies a culture was isolated which proved identical with *B. areoideae* Towns., and one of the female flies yielded a culture

differing from *B. aeroideae* only in its fermentation of dextrose. Both of these cultures produced rot in cabbage leaves and cabbage plants.

A number of puparia had been reserved for tests for the survival of the pathogene during the winter. Some of these were kept in slightly moistened sand at room temperature, while others were coverd with soil and left outdoors. In February plates were made from the contents of puparia from both lots. Seven out of 12 kept at room temperature contained colonies of *B. carotovorus,* and the same organism was isolated from 11 of the puparia exposed to freezing temperature. Apparently, the soft-rot pathogene is able to survive in the overwintering stage of the insect in Minnesota and can withstand winter temperature here.

Other puparia from the two lots were left undisturbed to await the emergence of the adult. These were used in an effort to determine if the parent of the spring brood can eliminate the soft-rot bacteria in the excrement. While many flies emerged, no excrement was obtained from any of them. However, the gastric tract was dissected out aseptically from many and was frequently found to contain *B. carotovorus.*

Although bacteria of various types, including the soft-rot pathogene, had been found in the meconium and the gastric contents of many of the adult flies, this does not prove the presence of the viable bacteria in the excrement. Accordingly, during the following summer, 74 puparia collected in the field were surface-sterilized, tested, and placed in dry sterile test tubes to allow the adults to emerge. Seventeen flies were obtained, 6 of which deposited excrement on the walls of the test tubes. After the removal of the flies to other sterile tubes, the excrement was moistened with a sterile cotton swab and inoculations made from it into broth from which agar plates were later poured. Bacterial growth developed on all the plates, and colonies of *B. carotovorus* were isolated from 3 of them. The same pathogene was obtained from the gastric contents of 2 of these flies. The 11 remaining flies were aseptically dissected. The gastric tracts so obtained were macerated in sterile tap water from which inoculations were made into cabbage-leaf sections. Soft rot developed from the inoculum of 5 of the flies. These experiments show that the soft-rot pathogene may survive in the gastric tract of the adult fly and that it may be excreted in viable form with the feces. This suggests that the adult also may be a factor in the dissemination of the soft-rot bacteria.

Tests for the presence of the soft-rot bacteria in the eggs.—Leach (11) found that the eggs of the seed-corn maggot could be surface-sterilized without injury to the embryo and that they are free from bacteria internally. It was thought possible that the same condition might obtain in the cabbage maggot. The eggs were first washed carefully to remove all adhering dirt particles. Then they were subjected to a solution of mercuric chloride

(1–1000) for 10 minutes, transferred to sterile broth to rinse off the disinfectant, and finally placed on sterile agar slants planted with surface-sterilized cabbage and radish seed. Three of the eggs floated on the solution and, when examined under the hand lens, were found to be mere shells. The tubes were examined frequently for contamination. Three of them developed a bacterial growth. Eight of the eggs did not hatch, probably having been injured in handling. In 49 of the sterile tubes the maggots crawled over the agar, but no bacterial growth developed in the tracks left by them on the agar, thus indicating that the eggs are free internally from bacterial contamination. The cabbage seedlings which had developed in the tubes during the incubation of the eggs were attacked by some of the larvae but developed no rot. Apparently, the larvae hatched from surface-sterilized eggs are free from bacteria, both internally and externally.

On the other hand, eggs placed without surface sterilization upon sterile agar slants produced bacterial growth on every slant. Bacteria from such growth when used as inoculum often produced soft rot in vegetables. Evidently, the eggs are contaminated externally with both saprophytic and pathogenic types of bacteria. Since it was previously found that the adult fly emerging from the puparium is smeared externally with these various bacteria, it is possible that the eggs may become contaminated in oviposition. It is also possible that such contamination may come from the soil, since, during the season, bacteria corresponding to *B. carotovorus* were isolated from soil samples from Crookston and St. Paul, Minnesota.

THE LARVA

As the newly hatched larvae struggle to free themselves from the egg shells, they may come in contact with the exterior of the shells and bacteria on the shells may thus be transferred readily to the exterior of the larvae. The soil may be a further source of contamination. Larvae hatched from eggs that were not surface-sterilized often produced soft rot in cabbage seedlings. Similarly, larvae hatched from surface-sterilized eggs were transferred to a growth of *B. carotovorus* for a short time, then removed to tubes containing cabbage seedlings; they soon reduced the seedlings to a rotting mass. Maggots were then collected from rotting cabbage heads in the field and placed upon cabbage leaves and seedlings. The large larvae approaching pupating conditions apparently did not feed upon the cabbage but pupated in a day or two and no rot followed. The smaller ones, evidently recently hatched, were very active and fed ravenously upon the food supply, soon bringing about a soft rot. Apparently, the larvae also become contaminated from various sources, the egg shell, the soil, or decaying plant material. The constant boring of the insect into the succulent tissues prevents these from healing over, and the contamination probably is trans-

ferred by the larvae, both from the mouth parts and the body in general to the fresh tissues thus constantly exposed. In this way the maggot serves as an agent of both dissemination and inoculation of the soft-rot pathogene.

PATHOGENICITY OF CULTURES ON CABBAGE PLANTS

Bacterial suspensions in water were made from the cultures obtained from the various stages of the insect. Inoculations were made from these into the stems and leaves of cabbage plants in the greenhouse. All the cultures produced soft rot, although some seemed more virulent than others. In the field it was much more difficult to obtain infection. Repeated inoculations were made, but only 12 per cent of the cultures caused a general collapse of the stems within 2 weeks. The same results were obtained in checks inoculated with *B. carotovorus* and other soft-rot cultures of known virulence. Since the weather was extremely dry, it is possible that a suberization of the tissue occurred before infection could take place. Stump rot developed in many of the inoculated plants later in the season. Among the noninoculated plants stump rot was much less prevalent, indicating that some of the rot appearing late in the season was due to the inoculations but was slow in developing. The same difficulty was experienced in the inoculations with the contents of the puparia and gastric tracts of adult flies, only about 15 per cent of the suspensions that had produced rot on cabbage plants in the greenhouse causing an immediate rot in the field. Again, stump rot was found in most of the inoculated plants later in the season, while very little was found in the noninoculated plants. Figure 1 shows the rot produced in a cabbage plant in the field as a result of inoculation with the contents of a puparium.

PATHOGENICITY ON POTATO STEMS

Inoculations were made with some of the cultures into potato stems and potato sprouts. A soft rot developed in both. While the decaying tissues of the cabbage did not change extremely in color, the decay in some of the potatoes was marked by a dark color varying in intensity according to the variety of potato used and resembling somewhat the discoloration found in potato stems affected with potato blackleg. Since Leach (11) has found that the seed-corn maggot serves as an agent of dissemination and inoculation of the potato blackleg, the idea might suggest itself that the flies from which these cultures were isolated were adult flies of the seed-corn maggot, especially as there is no known morphological difference between the female flies of the two species and both species attack cabbage. However, some of these cultures were isolated from the male fly, and this, as previously mentioned, is readily distinguished from the male fly of the seed-corn maggot.

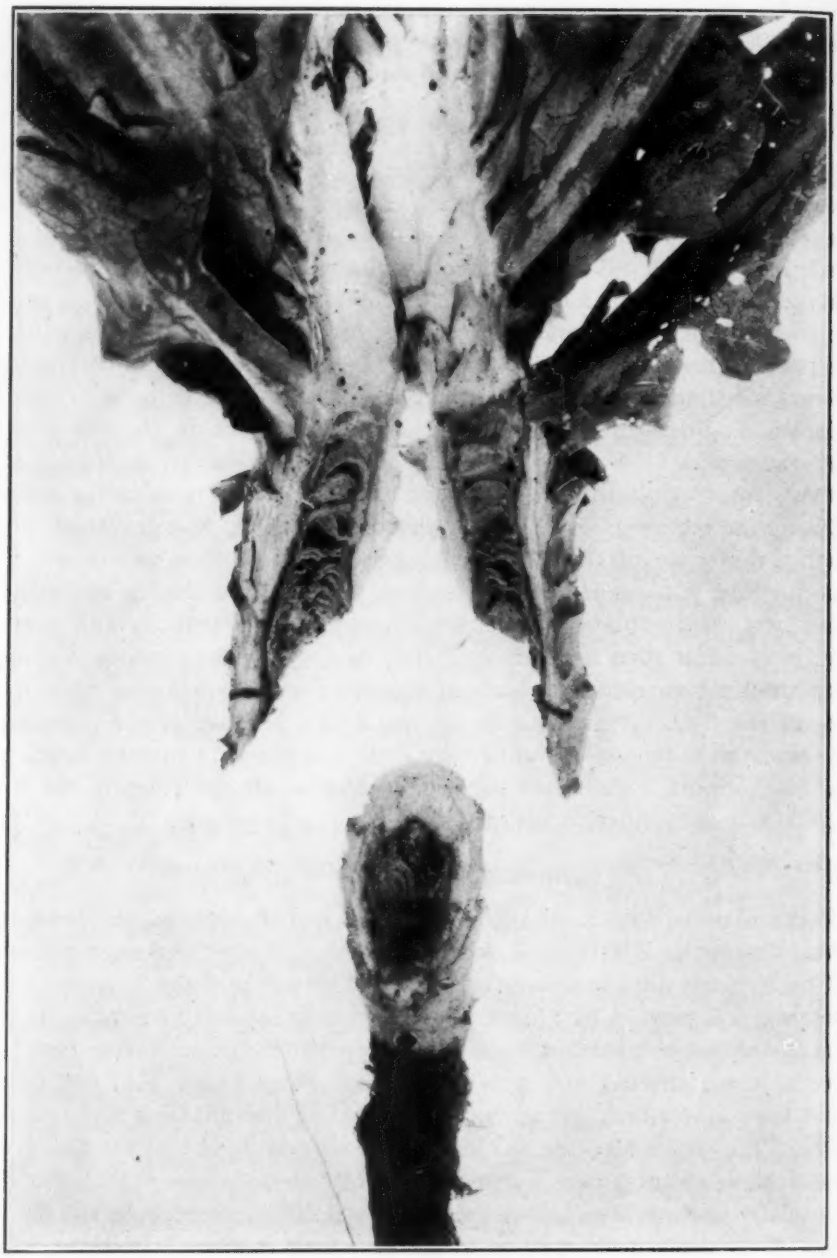

FIG. 1. Stump rot in cabbage developing in the field from inoculation with the contents of a surface-sterilized puparium of a cabbage maggot.

PATHOGENICITY OF B. CAROTOVORUS ON CAULIFLOWER AND OTHER CRUCIFEROUS PLANTS

Different conclusions have been reached by various investigators as to the pathogenicity of *B. carotovorus* on cauliflower and some other Cruciferae. Massey (13) reported, in 1924, that he was unable to produce a soft rot on cauliflower and kohl-rabi with *B. carotovorus*. Jones (10) had originally obtained the same results with his own culture of *B. carotovorus*. However, Harding and Stewart (8) found in 1902 that a culture isolated by them, as well as Jones' culture of *B. carotovorus*, produced a virulent soft rot on both cabbage and cauliflower plants.

The writer inoculated cauliflower heads with tap-water suspensions made from young agar cultures of *B. phytophthorus* Appel, *B. aeroideae* Towns., *B. carotovorus* Jones, and three other cultures of *B. carotovorus* of known virulence. Each of them reduced the cauliflower heads to a rotten mass in a few days. Inoculations were made also into cauliflower, turnip, and kohl-rabi with cultures isolated from the various stages of the cabbage maggot and identified as *B. carotovorus*, and soft rot was produced in all cases. Old cultures in broth were then used for inoculations but with negative results. When these cultures had been rejuvenated by transfer in broth a number of times, soft rot was obtained. Although the old broth cultures used for inoculation were cloudy and seemed to contain a heavy growth of the organism, probably only a few of the bacteria were in a viable condition and were unable to attack the vegetables inoculated before suberization of the tissues took place.

FEEDING EXPERIMENTS WITH LARVAE

According to Riley and Johannsen (14), Celli of Italy, in 1888, fed flies on pure cultures of *B. typhosus* and recovered the organism from the feces and intestinal tract of the insects. Cao (4), another Italian investigator, in 1906, starved cockroaches for 45 days, then fed them with bacteria of the colon-typhoid group and isolated the same type of bacteria from the excrement. Graham-Smith (7) conducted similar experiments on flies in 1910. In 1911 Bacot (1) reared larvae from *Musca domestica*, fed them with cultures of *B. pyocyaneus*, and isolated the organism from the digestive tract of both larvae and imagines. He concluded that pathogenic bacteria may be ingested by the larvae and persist in the adult, with some danger of being distributed in this way.

In the experiments on the cabbage maggot discussed in this paper it seemed apparent that the bacterial flora harbored by the insect varied according to the condition of the food ingested. Therefore, a study of this relationship was undertaken. Eggs were surface-sterilized and placed on sterile slants containing sterile, sprouting cabbage seeds. In a few days a

culture of a fluorescent bacterium was added. The eggs hatched and the larvae grew normally and pupated. The puparia thus obtained were surface-sterilized and the contents withdrawn with a sterile pipette and plated on agar. A high bacterial count of the fluorescent organism was obtained in all of them. Other eggs were placed upon surface-sterilized cabbage leaves, which were then inoculated with *Pseudomonas campestris* (Pam.) E. F. S. This organism also was recovered from the puparia which developed from the larvae. In the same manner, eggs were allowed to hatch on cabbage-leaf sections in petri dishes inoculated with cultures of a fluorescent and a yellow pigmented bacterium. Plates from the puparia obtained showed colonies of both types of bacteria. Similarly, larvae were reared from eggs on cabbage slices inoculated with *B. carotovorus*, and this organism was recovered from the resulting puparia. Bacterial counts made from the puparia in the latter group varied in proportion to the degree of the decomposition of the food consumed. It evidently can be concluded that the bacterial contents of the gastric tract of the larvae depend upon the type of food ingested. The tendency of such bacteria to persist throughout the life cycle of the insect makes it seem probable that the soft-rot pathogene will persist from generation to generation in the cabbage maggot.

OTHER BACTERIA ISOLATED FROM THE INSECT

As previously mentioned, a culture corresponding to *B. aeroideae* was isolated from an adult fly. Another organism, differing from the former only in its fermentation of dextrose, was obtained. Since a single-cell isolation was not made, it is possible that the latter culture may have been contaminated with a dextrose-fermenting organism that could not be removed by repeated platings. Both cultures produced a soft rot on cabbage plants in the greenhouse.

Fluorescent bacteria were always obtained from the insect. These consisted of two types, one a straight, stout, rod-shape organism; the other, slender and curved. Within each of these types variations occurred in the fermentation of various sugars. One of the cultures was identified as *Pseudomonas fluorescens* (Flügge) Migula. Another fluorescent organism, not definitely identified, produced a "water soaked" appearance in cabbage leaves, after which the tissue dried out. This occurred only under high temperature and humidity, so it is probably of no importance economically, since such conditions would not normally exist in storage.

Several times a spore-bearing bacterium, corresponding to *B. mesentericus* Flügge was obtained from puparia. Under conditions of high temperature and high relative humidity it caused a soft rot on cabbage leaves. But, since such conditions usually do not prevail in storage, the organism is probably of no importance in this respect. It did not affect the stems

of cabbage plants in the greenhouse. This bacterium is of some interest, since Brierly (3) has recently reported finding a strain of *B. mesentericus* that is pathogenic for potatoes but does not infect the stems of cabbage seedlings.

Various types of cellulose bacteria were isolated from a number of puparia collected from badly decayed cabbage heads. These included the yellowish pigmented strains of *Spirochaeta cytophaga* (Merker) Hutchinson and Clayton. This was anticipated, for cellulose bacteria were found on the exterior of sound vegetables and were very numerous in decayed cabbage. They probably are ingested in large numbers by the maggot. A yellow pigmented bacterium corresponding to *Ps. campestris* was isolated from the maggot many times. While the cultures showed some pathogenicity for cabbage plants in the greenhouse and may possibly indicate that the cabbage maggot may disseminate and inoculate the organism causing black rot of cabbage, the data obtained are insufficient to justify conclusions at this time.

OTHER INSECTS DISSEMINATING THE SOFT-ROT BACTERIA

Hylemyia antiqua Mg.—Smith and Dickerson (17), in 1907, made the following statement in regard to the onion maggot and its connection with the soft rot of onion: "In feeding, the maggots may attack the onion at the side, but generally they work upward through the center of the bulb, beginning at the bottom, especially when the onion is small. This they can readily do, because the tissue is rather soft and still further softened by the decay caused by the maggot injury." Gibson and Treherne (6) speak of the maggots working "their way down within the sheaths until they reach the young forming bulbs in which they feed and ultimately reduce the same, especially the lower portions, to a rotten mass."

In the course of the research reported here 23 puparia of the onion maggot were successfully surface-sterilized, and crushed in broth. Inoculations were made from 12 of these into onion bulbs and 9 of them produced a soft rot. Plates were made from the remaining 11 puparia and cultures of *B. carotovorus* were obtained from 4. The entire bacterial growth was washed from the 7 plates from which no soft-rot colonies had been isolated; inoculations were made from these suspensions into onion bulbs. Two of them produced the soft rot. Eight puparia were surface-sterilized and placed on sterile agar slants. The tracks left on the agar by the emerging adult flies became outlined by a bacterial growth, and cultures of *B. carotovorus* were obtained from 5 of them.

It is evident from this preliminary investigation that the onion maggot is a factor in the development of the bacterial soft rot of onion. In fact, it is very probable that all of the species of this group of insects will be

found to harbor the soft-rot pathogene and to serve as an agent of dissemination and inoculation of these bacteria.

Cynipids.—Parasites on the cabbage maggot were found to be numerous at the time of this investigation. From 214 puparia, planted on agar slants during the first year of this work, only 104 flies emerged. The rest of the puparia were parasitized by a Cynipid. Although no colonies of the soft-rot organism were found on the plates made from the gastric contents of these parasites, typical soft rot was produced in cabbage plants in the greenhouse from the inoculation of the macerated intestinal tract of many of these insects. The parasite probably had ingested the soft-rot bacteria in feeding upon its host and had retained these in viable form.

Staphylinids.—The puparia of the spring brood of cabbage maggots in 1928 were severely parasitized by a Staphylinid, the parasite emerging from 94 per cent of 494 puparia. The majority of these were *Baryodma bimaculata* Grvh., although several other types, not identified, were found. Four of the parasites emerged from the puparia while observed by the writer. The meconium of these empty puparia was swabbed out and inoculations were made into broth and plated. *Bacillus carotovorus* was isolated from 2 of them. Inoculations from the macerated gastric tract of some of the insects produced soft rot on cabbage plants. The Staphylinid apparently consumes every trace of the contents of the puparium and undoubtedly ingests the soft-rot bacteria present in the pupating maggot, many of these remaining viable in the parasite. Adult Staphylinids emerging from puparia often were found to harbor the soft-rot bacteria. This is probably of no economic importance, since the insect evidently does not attack the plant. Any dissemination of the pathogene it may bring about as a mechanical carrier is undoubtedly limited, while the heavy mortality it causes among the maggots indicates that it is a parasite of considerable importance.

Collembola spp.—Various species of Collembola were frequently found in rotting cabbage. Some of them were transferred to cabbage seedlings growing in sterilized soil, and decay followed their attacks upon the young plants. No attempts were made to ascertain if these insects harbor the soft-rot pathogene in the digestive tract, but they probably serve as mechanical carriers of various bacteria, including those of soft rot. This dissemination may be of no economic importance.

CONCLUSIONS

The cabbage maggot is often associated with the soft rot of cabbage and other vegetables. The egg is free from bacteria internally but is contaminated externally with various types of bacteria from such sources as the soil, decaying plant tissue, and probably the exterior of the parent fly. This contamination often includes the soft-rot bacteria.

The newly hatched larva is also contaminated on the exterior from somewhat similar sources, such as decaying plant material, the soil, and probably the exterior of the eggshell. In feeding upon the cruciferous plants, the maggot inoculates these bacteria, often including the soft-rot pathogene, into the succulent tissues of the host plant, and soft rot may thus develop in varying degrees simultaneously with the attack of the insect upon the plant. In this manner the larva may ingest decaying food tissue containing the soft-rot organisms. Many of these bacteria remain viable in the digestive tract of the maggot.

These organisms also persist in varying numbers inside the puparium and are voided in the meconium in sufficient numbers to contaminate the emerging adult fly. The same type of bacteria survive in the intestinal tract of the fly and are sometimes found in the feces. There is, therefore, a close association between the soft-rot pathogene and the cabbage maggot throughout its entire life cycle. While only a few of the maggots feeding on radishes with no visible decay were found to harbor the soft-rot bacteria, it is probable that the larvae, feeding upon the more soft-rot-susceptible cruciferous plants, ingest enough of these organisms to become an important factor as agents of dissemination and inoculation of the soft-rot bacteria.

In view of these facts it appears that it will be necessary to take into consideration the control of the cabbage maggot in planning measures of control of the soft rot of cruciferous plants.

SUMMARY

1. Entomologists and plant pathologists often have observed a close association between the lacerations of the cabbage plant by the cabbage maggot and the development of bacterial soft rot.

2. A study was made to determine the possibility of the dissemination and inoculation of the soft-rot bacteria by the maggot.

3. The eggs of the insect were found to be free from bacteria internally but contaminated externally with bacteria of various kinds, often including the soft-rot organisms. Such contamination probably is from the body of the parent fly, the soil, or decaying vegetable material.

4. The larvae hatched from surface-sterilized eggs contained no bacteria. However, they apparently become contaminated externally from various sources, such as the soil, decaying plant material, and probably from the contaminated eggshell. They ingest bacteria with their food.

5. The puparia contained viable bacteria of many types, including the soft-rot pathogene.

6. Such bacteria were found also in viable form in the intestinal tract and the excrement of the adult fly.

7. Soft-rot bacteria were found in overwintering puparia and were viable in puparia exposed to freezing temperatures.

8. There is evidently a close association between the soft-rot pathogene and the cabbage maggot in its entire cycle.

9. Cabbage-maggot larvae that had been feeding upon decaying cabbage tissue inoculated fresh cabbage leaves with soft-rot bacteria which reduced them to a rotten mass. The constant lacerations from the maggot prevented the wounded tissue from healing over and checking the decay.

10. Evidently, the larvae of the cabbage maggot, as well as those of closely related species, may serve as agents of dissemination and inoculation of the soft-rot bacteria.

11. Certain insects parasitic on the cabbage maggot were found to disseminate the same type of bacteria, but they are probably of little economic importance in this respect.

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HOST SPECIALIZATION OF BARLEY LEAF RUST, PUCCINIA ANOMALA

E. B. MAINS[1]

INTRODUCTION

Puccinia anomala Rostr. is a rust resembling in many respects the leaf rusts of wheat, *Puccinia triticina* Eriks., and rye, *Puccinia dispersa* Eriks. and Henn. It differs most noticeably from these in that a majority of its teliospores are unicellular, as in the genus Uromyces. Since, however, a small proportion of its teliospores are bicellular it is placed in the genus Puccinia. In other respects it resembles *Puccinia rubigo-vera* DC., having globoid or ellipsoid urediniospores, with scattered germ pores and the telia long covered by the epidermis of the host.

Various other names have been applied to this rust. The name *Puccinia simplex* (Keke.) Eriks. and Henn. (4) is often used. In the North American Flora, Arthur and Fromme (1) have listed it under the name, *Dicaeoma anomala.*

Compared with the other cereal rusts, this species has received very little study. In Russia, Tranzschel (11) has shown that it produces its aecial stage on *Ornithogalum umbellatum* L. and *O. narbonense* L. He was unable to infect *Muscari botryoides* (L.) Mill., *M. tenuiflorum* Tausch., *Scilla sibirica* Andr. and *Allium angulosum* L. In the United States, Mains and Jackson (8) were able to produce aecia on *O. umbellatum* but not on *Nothoscordium bivalve* (L.) Britton. The aecial stage has been produced also on *O. umbellatum* in Austria (2), Germany (6), and in France (3).

Very little study has been made of the specialization of this rust to grass hosts. For North America, Arthur and Fromme (1) report it only on *Hordeum vulgare* L. The Sydows (10) list it on *H. distichum, H. hexastichum, H. secalinum, H. vulgare,* and *H. zeocrithon.* In Germany, Klebahn (5) observed that in the field *H. distichon, H. distichon nigricans, H. hexastichon, H. zeocrithon, H. coeleste trifurcatum, H. vulgare,* and *H. vulgare cornutum* were heavily rusted, while *H. vulgare nigrum* was free from rust.

Vavilov (12) studied 82 agronomic varieties of barley for susceptibility to *Puccinia anomala.* Five varieties of *Hordeum vulgare hexastichum* L. were more or less susceptible (type 3 or 4). Thirty-nine varieties of *H.*

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vulgare tetrastichum Keke. were more or less susceptible (type 3 or 4), while 5 were somewhat resistant (type 2½) and one moderately resistant (type 2). Thirty-four varieties of *H. distichum* L. were more or less susceptible, 2 somewhat resistant, 3 moderately resistant, and 1 resistant (type 1).

In Australia, Waterhouse (13) has recently reported studies in which *Hordeum spontaneum* Koch was found to be completely susceptible (type 4). *Hordeum murinum* L. was very resistant (type 0). In a study of 116 varieties of barley, he found 54 varieties of *H. vulgare* L. susceptible (type 4), 10 moderately resistant (type 2), and 15 resistant (types 0–1). In *H. intermedium* Keke., all eight of the varieties studied were susceptible. In *H. distichon* L., 24 varieties were susceptible and one was resistant. In *H. deficiens* Steud., the four varieties studied were all susceptible.

RESULTS

The studies here reported were begun in 1918. The results obtained in a study of the aecial hosts have already been reported in a previous paper (8). The occurrence of physiologic forms in this rust also has been briefly noted (7). This paper gives in more detail the results obtained in a study of its host specialization.

Wild grass hosts. During the first few years of work on barley leaf rust, attention was devoted largely to a study of the susceptibility of grasses to this rust. In this study 25 to 35 seedlings of several grasses were inoculated in the greenhouse with a number of collections of the leaf rust of barley.

In 1918, a collection of rust obtained by Prof. H. S. Jackson at Ithaca, N. Y., was sown on seedlings of *Agropyron cristatum*[2] Beauv., *A. repens* (L.) Beauv., *A. tenerum* Vasey, *Bromus inermis* Leyss., *B. pratensis* Lam., *B. secalinus* L., *B. sitchensis* Bong., *B. sterilis* L., *B. tectorum* L., *B. villosus* Forsk., *Elymus australis* Scribn. & Ball, *E. canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl., *Hordeum distichon* L. (Hanna, Hannchen), *H. jubatum* L., *H. pusillum* Nutt., *H. vulgare* L. (Tennessee Winter), *Hystrix hystrix* (L.) Millsp., *Puccinellia nuttalliana* (Schultes) Hitch., *Sitanion hystrix* (Nutt.) J. G. Smith, and *Triticum vulgare* Vill. (Gypsy, Fultzo-Mediterranean). Uredinia were produced in abundance on *Hordeum distichon* and *H. vulgare*. No signs of infection other than occasional faint flecks were noted on the other grasses. This series of grasses was reinoculated twice during the winter with similar results.

Another collection of the leaf rust of barley made by Prof. H. S. Jackson at Madison, Wis., November, 1918, was sown on seedlings of *Agropyron caninum* (L.) Beauv., *A. cristatum*, *A. pseudorepens* Scribn. & Smith, *A.*

[2] Thanks are due Prof. A. S. Hitchcock for identifying and checking the determination of the grasses studied.

repens, A. Smithii Rydb., *A. tenerum, Alopecurus geniculatus* L., *A. pratensis, Bromus altissimus* Pursh., *B. carinatus* Hook. & Arn., *B. erectus* Huds., *B. hordeaceus* L., *B. inermis, B. japonicus* Thunb., *B. Kalmii* A. Gray, *B. polyanthus* Scribn., *B. pratensis, B. secalinus, B. sitchensis, B. tectorum, B. tectorum nudum* Mert. & Koch., *B. villosus, Elymus australis, E. canadensis, E. glaucus, E. virginicus* L., *Festuca elatior* L., *F. rubra* L., *Hordeum distichon* (Chevalier, Hanna), *H. jubatum, H. murinum* L., *H. nodosum* L., *H. pusillum, H. vulgare* (Odessa, Tennessee Winter), *Hystrix hystrix, Puccinella* sp., *Panicularia borealis* Nash., *P. grandis* (S. Wats.) Nash., *Poa pratensis* L., *P. triflora* Gileb; *Sitanion hystrix, Secale cereale* L. (Mammoth Winter), and *Triticum vulgare* (Gypsy, Fultzo-Mediterranean). Uredinia were produced only on varieties of *Hordeum distichon* and *H. vulgare.* No signs of infection were noted other than occasional faint flecking on the other grasses. This series of grasses was reinoculated twice during the winter with similar results.

In 1919, a collection of leaf rust made by the writer at La Fayette, Ind., was sown on seedlings of *Agropyron cristatum, A. tenerum, Elymus canadensis, E. glaucus, Hordeum distichon* (Hanna, Hannchen), *H. pusillum, H. vulgare* (Manchuria, Mariout), *Hystrix hystrix, Sitanion hystrix, Puccinellia* sp., and *Triticum vulgare* (Red Wave). Uredinia developed only on the varieties of *Hordeum distichon* and *H. vulgare.* Slight flecking showed on *Secale cereale, Triticum vulgare,* and *E. canadensis.*

In 1920, leaf rust of barley collected by E. Bethel at San Diego, California, was sown on seedlings of *Hordeum distichon* (Hanna), *H. murinum, H. nodosum, H. pusillum,* and *H. vulgare* (Tennessee Winter). Abundant uredinia were produced on the varieties of *H. distichon* and *H. vulgare,* while only a few faint flecks developed on the other grasses.

A collection of the leaf rust of barley made by the writer at La Fayette, Ind., was sown on seedlings of *Hordeum boreale* Scribn. & J. G. Smith, *H. caespitosum* Scribn., *H. distichon* (Hanna), *H. gussoneanum* Parl, *H. jubatum, H. maritimum* With., *H. murinum, H. nodosum, H. pusillum,* and *H. vulgare* (Oderbrucker, Beardless, Manchuria). Abundant uredinia developed on the varieties of *H. distichon* and *H. vulgare.* The only signs of infection on the other species of Hordeum were faint flecks.

In 1921, a collection of leaf rust of barley made by H. B. Humphrey at Mt. Vernon, Washington, was sown on seedlings of *Hordeum deficiens* (Blackhull), *H. distichon* (Chevalier, Hannchen), *H. gussoneanum, H. intermedium* (Nakano Wase), *H. murinum, H. nodosum, H. pusillum, H. secalinum* Schreb., *H. silvaticum, H. spontaneum* C. Koch, and *H. vulgare* (Manchuria, Tennessee Winter). Abundant uredinia developed on *H. spontaneum* and the varieties of *H. distichon, H. intermedium,* and *H. vulgare.* The other species showed only occasional faint flecks.

These studies would, therefore, indicate that the leaf rust of barley is probably limited to the closely related species *Hordeum deficiens*, *H. distichon*, *H. intermedium*, *H. vulgare*, and *H. spontaneum*.

Physiologic forms of the rust differentiated by barley varieties.—Attention was next directed to a study of the relative susceptibility of barley varieties. Through the kindness of H. V. Harlan and M. N. Pope, of the Office of Cereal Crops and Diseases, an extensive series of over 600 barley varieties was received for study. During the winter of 1923–24, these varieties were given a thorough test in the seedling stage in the greenhouse with a culture of leaf rust received from Washington, D. C. To this collection most of the varieties of *Hordeum distichon*, *H. intermedium*, *H. vulgare*, and *H. deficiens* proved to be susceptible. However, a number of varieties especially of *H. vulgare* and *H. distichon* showed resistance. A selected series of these varieties has been studied in succeeding years with other collections of the rust. As a result of these studies, it was found that two physiologic forms of the rust occurred in the United States that could be distinguished by the reactions of certain of these varieties.

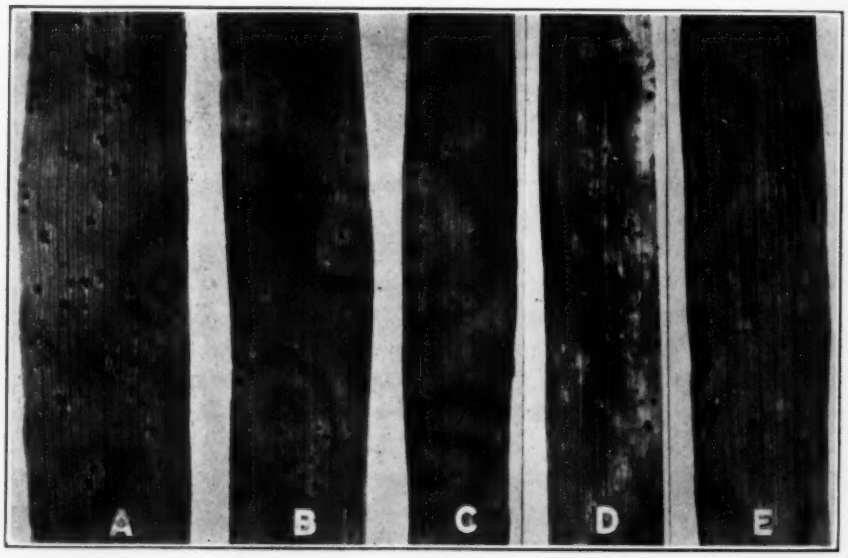

FIG. 1. Types of reaction to leaf rust, physiologic form 1. A, susceptible, type 4, uredinia large, numerous, little or no chlorosis (Wisconsin Winter 49); B, moderately susceptible, type 3, uredinia moderate to large, more or less numerous, usually some chlorosis (Eagle C. I. 913); C, moderately resistant, type 2, uredinia moderate in size, chlorotic and necrotic spots prevalent, frequently without uredinia (Limerick C. I. 1302); D, very resistant, type 1, uredinia small, few, infection mostly evident as necrotic spots without uredinia (Peruvian C. I. 935); E, highly resistant, type 0, no uredinia produced, infection evident only as small necrotic spots or flecks (Horsford C. I. 877).

It is not proposed in this paper to attempt to give all the data accumulated in this study. However, a set of seventeen varieties has been selected which, it is believed, best characterize the two physiologic forms of the rust. Nine varieties which show marked differences in reaction to the two forms, 1 variety susceptible to both physiologic forms, and 7 varieties highly resistant to both physiologic forms have been included.

The methods were similar to those used by Mains and Jackson (9) in their study of the leaf rust of wheat, *Puccinia triticina*. Inoculations were made on seedlings in the second- to third-leaf stage. All studies were made in the greenhouse of Purdue University Agricultural Experiment Station during the winter months.

Five classes of reaction were recognized: Susceptible was denoted by 4 (Fig. 1, A); moderately susceptible, by 3 (Fig. 1, B); moderately resistant, by 2 (Fig. 1, C); very resistant, by 1 (Fig. 1, D); highly resistant, by 0 (Fig. 1, E). These agree with the classes used by Waterhouse (13) in his studies of this rust and also by Mains and Jackson (9) for other leaf rusts of the cereals.

The results of these studies are given in table 1. As is evident from this table, two physiologic forms have been sharply differentiated by the

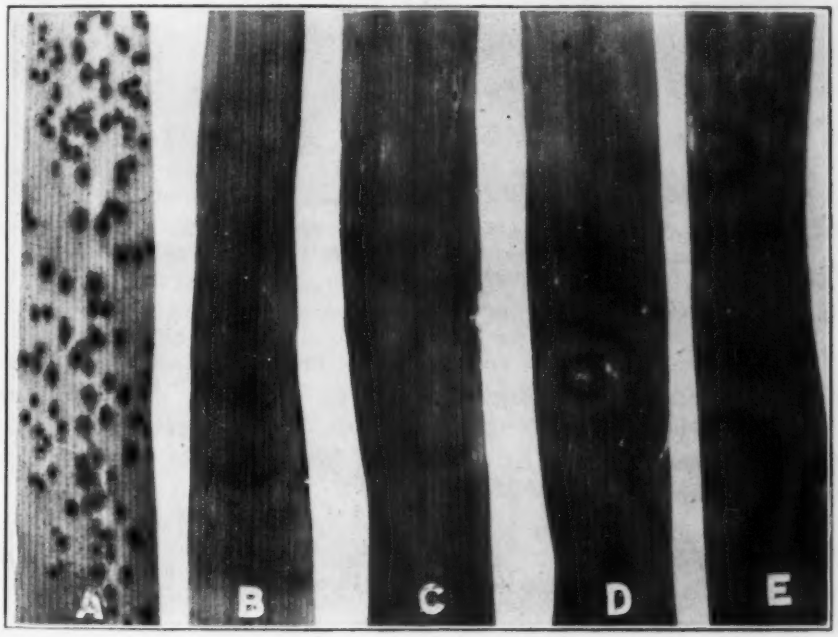

FIG. 2. Reaction of barley varieties to physiologic form 1 of *Puccinia anomala*. A, susceptible, Hanna 906; B, resistant, Beardless C. I. 610; C, Horsford C. I. 507; D, Oderbrucker C. I. 940, and E, Bolivia C. I. 1257. Compare with reaction to physiologic form 2 (Fig. 3).

reaction of Featherston C. I. 1120, Oderbrucker C. I. 940, Oderbrucker C. I. 957, Unnamed C. I. 1347, Malting C. I. 1129, Manchuria C. I. 2330, Hooded Spring C. I. 716, Horsford C. I. 507, and Horsford C. I. 877 (Figs. 2 and 3). These have all been highly resistant to physiologic form 1 and susceptible to physiologic form 2.

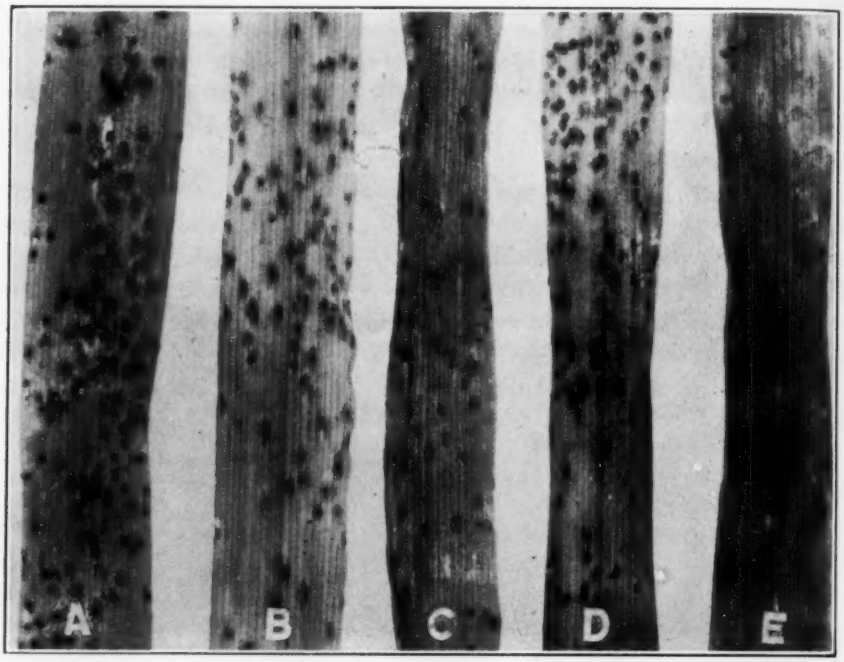

FIG. 3. Reaction of barley varieties to physiologic form 2 of *Puccinia anomala*. A, susceptible, Hanna C. I. 906; B, Beardless C. I. 610; C, Horsford C. I. 507; D, Oderbrucker C. I. 940; E, resistant, Bolivia C. I. 1257. Compare with the reaction to physiologic form 1 (Fig. 2).

Physiologic form 1 was collected as follows:[3] 1923, Arlington, Va.; 1924, Knoxville, Tenn., Arlington, Va.; 1925, Arlington, Va.; 1927, Hubbard, Iowa, Mecklenburg, N. Y., Arlington, Va., La Fayette, Ind.; 1928, San Juan Island, Wash., Atlantic, Iowa, Maumee, Ohio; 1929, Denton, Texas, La Fayette, Ind.

Physiologic form 2 has been obtained from the following localities: 1925, Knoxville, Tenn.; 1927, Knoxville, Tenn., Marshall, N. C., Manhattan, Kans., MacDonald College, Canada; 1928, Arlington, Va., Knoxville, Tenn., Maumee, Ohio, Lewisburg, Ohio.

[3] The writer wishes to express his thanks to H. B. Humphrey, C. E. Leighty, P. W. Rohrbaugh, J. W. Baringer, and C. O. Johnston for their kindness in supplying collections of the rust employed in these studies.

TABLE 1.—*Reaction of seventeen varieties of barley to two physiologic forms of leaf rust, Puccinia anomala*

Variety	C. I. No.[a]	B. P. No.[b]	Physiologic form 1[c]							Physiologic form 2				
			1923	1924	1925	1926	1927	1928	1929	1925	1926	1927	1928	1929
Featherston	1120	320	0	0	0–1	0–1	0–1	0–1	0–1	4	3–4	4	3–4	3–4
Oderbrucker	940	182	1	0	0–1	0–1	0	0–1	0–1	4	3–4	3–4	4	3–4
Oderbrucker	957	193	1	0	0	0–1	0	0–1	0–1	4	3–4	4	3–4	4
	1347	437	0	0	0	0	1	1	0–1	4	4	4	4	4
Malting	1129	326	1	0	0	0–1	0	0	0–1	4	3–4	3–4	3–4	4
Manchuria	2330	538	0	0	0	0	1		0–1	4	4	4–		4
Hooded Spring	716	121	0	1	0–1	0–1	0–1	0	0–1	4–	4	3–4	3–4	4
Horsford	507	48	0	0	0	0–1	0	0	0–1	4	3–4	4	3–4	4
Horsford	877	147	0	0	0–1	0–1	0	0	0–1	4	3–4	3–4	3–4	4
Hanna	906	153	4	4	4	4	4	4	4	4	4	4	4	4
Callas	2440	604	2	1	1	0–1	0–1	0–1	0–2	1	1	1	0–1	1–2
Mecknos Moroc	1379	463	1	0	0	0	1	0–2	0–1	0	0–1	1	0–1	1–2
Peruvian	935	178	1	1	0–1	0–1	0–2	0	0–1	0–1	1–2	1–2	0	0–2
Quinn	1024	247	2	0	0	0	0–1	0	0–2	0	0	0	0	0–1
Bolivia	1257	375	1	0	0	0	0	0	0	0	0–1	0	0	0–1
Juliaca	1114	314	0	0	0–1	0	0–1	0–2	0–1	0	0–1	0	0	0–1
	2329	537	2	1	1	0–1	0–1	0–1	0–1	0	0	0	0–1	0–1

[a] Accession number of the Office of Cereal Crops and Diseases of the U. S. Department of Agriculture.

[b] Accession number of the Department of Botany, Purdue University Agricultural Experiment Station.

[c] Rust collected during the summer of the years listed below and studied during the following winter months. The results for all collections of each year are summarized in each column.

A mixture of the two physiologic forms occurred in only one collection, that from Maumee, Ohio, in 1928. Collections from La Fayette, Ind., have not been included for the most part, since cultures of the two forms have been introduced into the experimental plots in studies of varietal susceptibility. Consequently, the forms present did not indicate natural distribution.

It is interesting to note the occurrence of physiologic forms at Arlington, Va., and Knoxville, Tenn. Physiologic form 1 was prevalent at Arlington, Va., in 1923, 1924, 1925, and 1927, while physiologic form 2 occurred in 1928. At Knoxville, Tenn., physiologic form 1 occurred in 1924, while physiologic form 2 was prevalent in 1925, 1927, and 1928.

Waterhouse (13), in his study of barley varieties for reaction to *Puccinia anomala*, in Australia, found that 16 out of 116 varieties showed considerable resistance and 10 were moderately resistant in studies of the seedlings during the winter months. The same author (14) has recently shown that during the summer months a number of these are susceptible.

TABLE 2.—*Reaction, to physiologic forms 1 and 2 of leaf rust in the United States, of barley varieties resistant in Australia*

Variety	Australian No.	B. P. No.	Physiologic form 1		Physiologic form 2		Australian[a] rust
			1928	1929	1928	1929	
California Feed	B59	799	4–	2–3	4	3	2
Psakwon	B81	800	4	4	4	4	2
Locride	B86	801	4	3	3–4	4	2
Sahara	B95	802	4	3	4	2	2
Coast	B121	803	4	4–	4–	3	2
Marionet	B113	804	4–	4–	2–3	3	2
Orge Fourrager	B102	805	1	1	2	1	2
Lion	B33	806	4	4	4	4	2
Smooth Awn × Luth	B34	807	4	4	4–	4	2
Smooth Awn × Manchuria	B35	808	4–	4	2	4	2
C. I. 2208		809	2	0	3	4	0–1
Virginia Hooded	B6	810	2+	1	3	2–3	0–1
Smooth Awn × Manchuria	B36	811	2	2	3+	3–4	0–1
Smooth Awn × Manchuria	B37	812	2+	2	3+	4	0–1
Smooth Awn × Manchuria	B38	813	2	1	3+	4	0–1
Manchuria Sel. C163	B40	814	2+	1	4	4	0–1
Manchuria Sel. C168	B41	815	2+	2	3+	4	0–1
Manchuria Minn. 184	B43	816	2+	1	3+	4	0–1
Manchuria	B44	817	2+	1	4	3–4	0–1
O. A. C. 21	B61	818	2+	2	4–	4	0–1
No. 22	B69	819	1–2	0	2	2	0–1
No. 305	B85	820	3	2–3	3	2	0–1
Colsess	B97	821	2	0	3–4	4	0–1
Orge 4th	B100	822	0	0	0	0	0–1
Orge 14 J	B101	823	0	0	0	0	0–1
C. I. 2220	B23	824	2+		4		0

[a] Reaction given by W. L. Waterhouse. Jour. Proc. Royal Soc. N. S. Wales **61**: 223–225. 1928.

Mr. Waterhouse kindly furnished the writer with seed of these 26 varieties and these have been studied for reaction to physiologic forms 1 and 2 during the winters of 1928–29 and 1929–30. The results of these studies are given in table 2 in comparison with those obtained by Waterhouse with the Australian rust under similar conditions.

The results would indicate that the rust of Australia is a third physiologic form. Most of the varieties were more susceptible to physiologic forms 1 and 2 than reported by Waterhouse. The Australian rust approached physiologic form 1 in that all the varieties highly resistant to it also showed some resistance to physiologic form 1, although to a less extent.

Four varieties, Orge Fourroger B102, No. 22 B69, Orge 4th B100, Orge 14J B101, were resistant to all of the forms studied in Australia and the United States. The last two were very resistant to both physiologic form 1 and 2 as well as the Australian rust.

SUMMARY

1. In these studies favorable hosts for the leaf rust of barley, *Puccinia anomala*, were found in only a few closely related species of Hordeum, viz.: *H. vulgare, H. deficiens, H. distichon, H. intermedium*, and *H. spontaneum.*

2. Seedlings of *Agropyron caninum, A. cristatum, A. pseudo-repens, A. repens, A. Smithii, A. tenerum, Alopecurus geniculatus, A. pratensis, Bromus altissimus, B. carinatus, B. erectus, B. hordeaceus, B. inermis, B. japonicus, B. pratensis, B. secalinus, B. sitchensis, B. sterilis, B. tectorum, B. tectorum nudum, B. villosus, Elymus australis, E. canadensis, E. condensatus, E. glaucus, E. virginicus, Festuca elatior, F. rubra, Hordeum boreale, H. caespitosum, H. gussoneanum, H. jubatum, H. maritimum, H. murinum, H. nodosum, H. pusillum, H. secalinum, H. silvaticum, Hystrix hystrix, Panicularia borealis, P. pratensis, P. grandis, Poa pratensis, P. triflora, Puccinellia nuttalliana, Secale cereale, Sitanion hystrix*, and *Triticum vulgare* showed little or no signs of infection beyond occasional slight flecking.

3. Two physiologic forms of *Puccinia anomala* have been distinguished in the United States. These are distinguished by the differences in reaction of a select set of barley varieties, Featherston C. I. 1120, Oderbrucker C. I. 940, Oderbrucker C. I. 957, Unnamed C. I. 1347, Malting C. I. 1129, Manchuria C. I. 2330, Hooded Spring C. I. 716, Horsford C. I. 507, and Horsford C. I. 877, which are highly resistant to physiologic form 1 and more or less susceptible to physiologic form 2.

4. Seven varieties, Callas C. I. 2440, Mecknos Moroc C. I. 1379, Peruvian C. I. 935, Quinn C. I. 1024, Bolivia C. I. 1257, Juliaca C. I. 1114, and unnamed C. I. 2329, were found to be more or less resistant to both physiologic forms.

5. Of a series of 26 varieties, found by Waterhouse to be resistant to *Puccinia anomala* in Australia, 22 varieties were more susceptible to the physiologic forms from the United States, indicating the existence of a third physiologic form in Australia.

6. Four varieties from Australia, Orge Fourrager B102, No. 22 B69, Orge 4th B100, and Orge 14 JB101, were resistant to the physiologic forms used in these studies as well as to the rust in Australia.

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STUDIES ON THE PROPERTIES OF THE BEAN-MOSAIC VIRUS

T. G. FAJARDO[1]

The determination of the properties of different plant viruses now occupies an increasingly important position with respect to their classification. The properties of the ordinary tobacco-mosaic virus and that of cucumber mosaic, for example, are now well known (7, 4), but, up to the present, nothing has been reported concerning certain properties of the bean-mosaic virus. This may be because bean mosaic is difficult to transmit artificially, a difficulty frequently encountered by the earlier workers. A method of artificial inoculation has been developed by the writer (5), however, that has given a fairly high percentage of infection and has made possible a preliminary study of the properties of this virus. The investigation has been conducted in relation to the following phases: (1) Effect of aging on the virus *in vitro*, (2) effect of dilution, (3) resistance to alcohol treatments, (4) resistance to drying in plant tissues, (5) thermal death point, (6) effect of dry heat on the virus in infected seeds, (7) effect of freezing, and (8) filterability through bacterium-proof filters.

METHODS

The methods followed in these studies were uniform unless otherwise stated. The bean-mosaic virus was obtained in plant extract either by grinding up mosaic bean plants in a sterilized mortar with sterile sand or by passing diseased material through a fine meat chopper. The juice was then separated from the macerated tissues by squeezing it through cheesecloth. After the various treatments, the juice was artificially inoculated to young healthy seedlings of the variety Rogers Stringless Green Pod or Refugee 1000–1, both of which are late susceptible varieties. The inoculation was performed by rubbing or mutilating the two simple leaves of the plants with a small piece of cheesecloth dipped into the juice, to which a small quantity of sterilized sand had been added. The control plants were inoculated by the same method with a portion of the same but nontreated plant extract. The plants were kept in a greenhouse at a temperature of 24–28° C. for 25 days. Under ordinary greenhouse conditions, infected plants develop typical mosaic symptoms 15 to 20 days after inoculation.

RESULTS

(1) *Effect of aging on the virus in vitro.* The results of a series of experiments have shown that the virus of bean mosaic loses its power of

[1] The writer is indebeted to Professor L. R. Jones, under whose encouragement the work was pursued, and to Drs. J. Johnson and I. A. Hoggan, who kindly read and criticized the manuscript during its preparation.

infection rapidly in plant extract. Undiluted juice, whether allowed to stand in bulk or in 5 c.c. portions at room temperatures, failed to cause infection after 24 hours. Juice diluted with sterile water under the same greenhouse conditions loses its infectiousness more rapidly. Dilutions of 1 part of plant extract to 10 parts of water remained infectious after 14 hours, while dilutions of 1 to 50 and 1 to 100 failed to produce infection after this time. The percentage of infection decreased markedly with increase in dilution and duration of aging.

(2) *Effect of dilution.* The results of four experiments indicate that the bean-mosaic virus loses its infectious properties readily on dilution with distilled water, without aging. A low percentage of infection was obtained when the virus was diluted in plant extract to 1 to 1000. No infection was secured at dilutions higher than 1 to 1000.

(3) *Resistance to alcohol treatments.* The virus of bean mosaic is very sensitive to alcohol. A low percentage of infection was obtained after treatment of the virus with 50 per cent alcohol at room temperatures for less than five minutes, while no infection resulted after treatment with 75 per cent alcohol or higher strengths for the same length of time. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes at room temperature.

(4) *Resistance to drying in plant tissues.* The bean-mosaic virus is also very sensitive to drying and loses its infectious properties rapidly on drying. Cut young mosaic plants were allowed to wilt or dry at room temperature for various lengths of time, each sample being weighed at the beginning and end of the period. The loss in weight was then replaced by addition of distilled water, the plants macerated, and the extract obtained inoculated immediately to young plants. Table 1 shows the results of two such experiments and indicates that the virus was still infectious in plants that had been allowed to dry for 48 hours. No infection was secured from plants after drying 72 hours. Young mosaic plants kept turgid in an ice box, however, were still infectious after 72 hours. The virus, therefore, lives longer in plant tissues than in plant extract but becomes inactivated when the tissues dry out.

(5) *Thermal death point.* The bean-mosaic virus has a relatively low thermal death point. Five and 10 c.c. portions of mosaic plant extract were heated for 10 minutes in thin-wall test tubes in a water bath. After heating at 44–46° C., a single infection resulted in 10 plants inoculated, while no infection obtained after heating at 55–56° C. The thermal death point for the virus of bean mosaic therefore appears to lie between 44° C. and 56° C., probably close to 46° C.

(6) *Effect of dry heat on the virus in infected seeds.* Several tests with mosaic seeds of the varieties Rogers Stringless Green Pod and Refugee

TABLE 1.—*Resistance of the bean-mosaic virus to drying at room temperature*

Inoculum	Duration of aging	Loss of weight	Plants infected of 5 inoculated
	Hours	*Per cent*	*No.*
Series I			
1 (Control)	0	0.0	5
2	4	35.7	5
3	12	53.6	4
4	24	66.7	1
5	48	80.0	1
6	72	82.6	0
7	96	86.4	0
Series II			
1 (Control)	0	0.0	4
2	16	50.0	4
3	24	65.0	3
4	48	84.0	1
5	72	87.5	0
6	96	90.0	0

1000–1, heated 12 and 24 hours, respectively, at various temperatures in thermo-regulated ovens, showed that the virus could withstand higher temperatures in dry seeds than in plant extract. A certain number of mosaic seedlings were obtained from seeds heated at 65–66° C. for 24 hours, while seeds heated at 80° to 85° C. failed to germinate. Water extracts of the seeds heated at 80°–85° C. gave no infection when inoculated to young bean plants. These results, summarized in table 2, show that the highest temperatures which allow of subsequent germination do not destroy the virus in the seed. This is in agreement with the conclusions reached by Reddick and Stewart (10).

(7) *Effect of freezing.* Freezing undiluted, freshly expressed juice of mosaic plants at temperatures of –7° to –10° C. did not appear to affect the viability of the virus. The percentage of infection was found to decrease with the duration of freezing, until after 24 hours no infection resulted. The results, however, were similar to those obtained when testing at room temperature the effect of aging on the virus. It is, therefore, believed that the freezing, itself, did not cause inactivation of the virus.

(8) *Filterability through bacterium-proof filters.* The bean-mosaic virus, as far as could be determined, failed to pass through any grade of Berkefeld filter at a reduced pressure. In these experiments, inoculation of the first 5 to 10 c.c. of the filtrate passing through a grade V, coarse Berkefeld filter gave no infection. The nonfiltered juice was found to be still highly infectious and gave 100 per cent infection.

TABLE 2.—*The effect of dry heat on the bean-mosaic virus in infected seeds*

Variety	Time and temperature of heating	Seeds planted	Germination	Mosaic infection
		No.	*Per cent*	*Per cent*
	12 Hours			
	24° C.	20	95	58
Rogers	44–46° C.	20	60	67
Stringless	65–68° C.	40	80	59
Green Pod	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	75	73
	44–46° C.	20	85	71
	65–66° C.	40	90	72
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	12 Hours			
	24° C.	20	90	94
Refugee	44–46° C.	20	85	88
1000–1	65–66° C.	40	58	82
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	95	84
	44–46° C.	20	90	83
	65–66° C.	40	25	60
	80–85° C.	20	0	0
	85–90° C.	20	0	0

A COMPARISON OF THE PROPERTIES OF THE BEAN-MOSAIC VIRUS WITH THOSE OF CERTAIN OTHER VIRUSES

These results indicate that bean mosaic is caused by a specific virus, distinct from other viruses that have been similarly studied, such as the virus of tobacco mosaic, of cucumber mosaic, and of potato-crinkle mosaic. This is readily seen from table 3. While the tobacco-mosaic virus may remain infectious in plant extract for 1 or more years (3), the bean-mosaic virus apparently retains its infectiousness only about 20–24 hours, in this respect resembling more closely the viruses of cucumber mosaic and potato-crinkle mosaic, which lose their power of infection in 24–48 hours and in 24 hours, respectively (4, 8). The virus of bean mosaic retains its infectious nature in dried mosaic tissues between 2 and 3 days; that of tobacco mosaic for more than 1½ years (1); and that of cucumber mosaic (4), less than 10 days.

Again, while the bean-mosaic virus gives no infection at dilutions higher than 1–1000, the tobacco- and cucumber-mosaic viruses are still infectious at dilutions of 1 to 10,000 (2, 4). On the other hand, the crinkle-mosaic virus produces little infection at dilutions above 1 to 10 (8) and is, in this respect, more sensitive than the bean-mosaic virus.

The bean-mosaic virus is relatively sensitive also to alcohol. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes, while the tobacco-mosaic virus resists 50 per cent alcohol for several days and 80 per cent alcohol for half an hour or less (3). Johnson (7) obtained no infection with the cucumber-mosaic virus after treatment with 50 per cent alcohol for 1 hour.

The viruses also differ in their thermal death points, that of bean mosaic lying between 44° and 56° C., probably close to 46° C., that of tobacco mosaic being about 90° C. (9), that of cucumber mosaic 75° C. (4), and that of potato-crinkle mosaic 43° C. (8).

No success was obtained in attempts to filter the bean-mosaic virus through coarse Berkefeld filters, the virus being in this respect comparable to that of crinkle mosaic. The tobacco-mosaic virus has been shown by Iwanowski (6) and others to pass readily through bacterium-proof filters, and the cucumber-mosaic virus has been shown by Doolittle (4) to pass through a medium Berkefeld filter.

TABLE 3.—*Comparison of the properties of the bean-mosaic virus with those of the viruses of potato, tobacco, and cucumber mosaics*

Property of virus	Source of virus			
	Bean mosaic	Potato "crinkle mosaic"[a]	Tobacco mosaic[b]	Cucumber mosaic[c]
Resistance to aging *in vitro*	20–24 hrs.	24 hrs.	1 or more yrs.	24–48 hrs.
Tolerance to dilution	1–1000	1–10	1–10,000	1–10,000
Resistance to alcohol	25% for 30 min.	——	80% for 30 min.	50% for 1 hr.[a]
Resistance to aging in dried plant tissues	48–72 hrs.	——	1 or more yrs.	Less than 10 days
Thermal death point	Probably close to 46° C.	43° C.	90° C.[d]	75° C.
Filterability through coarse Berkefeld filter	Not filterable	Not filterable	Filterable	Filterable

[a] Determination by Johnson.
[b] Determination by Allard except as noted.
[c] Determination by Doolittle except as noted.
[d] Determination by Mulvania.

SUMMARY

(1) The virus of bean mosaic differs in its properties from the viruses of tobacco mosaic, cucumber mosaic, and potato-crinkle mosaic.

(2) The bean-mosaic virus retains its infectiousness for 20–24 hours in plant extract, when aged at room temperatures. In diluted juice, the virus loses its infectiousness sooner than in nondiluted juice.

(3) At a dilution of 1–1000 with distilled water, mosaic-plant extract gives a low percentage of infection, but at higher dilutions no infection has been obtained.

(4) The virus is inactivated by treatment with 25 per cent alcohol for 30 minutes and with 75 per cent alcohol for less than 5 minutes.

(5) The virus is very sensitive to drying in plant tissues, since infection was obtained from mosaic seedlings that had been allowed to dry at room temperatures for 72 hours.

(6) The thermal death point of the virus appears to lie between 44° and 56° C., probably close to 46° C.

(7) The virus is able to resist higher temperatures when in the seed than in expressed juice. It was not destroyed at temperatures that did not destroy the germinating capacity of the seed.

(8) Freezing apparently does not affect the viability of the virus.

(9) The virus was not found to pass through any grade of Berkefeld filter.

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REPORT OF THE COTTON-ROOT-ROT CONFERENCE AT TEMPLE, TEXAS

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The third annual conference of workers engaged in the study of cotton root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, was called by Director A. B. Conner of the Texas Agricultural Experiment Station at the Kyle Hotel, Temple, Texas, on January 15, 1930. Director Conner presided at the morning session and Dr. A. G. McCall, Chief of Soil Investigations, Bureau of Chemistry and Soils, U. S. Department of Agriculture, at the afternoon session. The meeting was attended by about 45 people, 12 of whom were from the U. S. Department of Agriculture, 15 from the Texas Agricultural Experiment Station, and the remainder press representatives and other nontechnical persons interested in the problem. Approximately 40 papers were presented; these are summarized below in the order of presentation.

Occurrence, losses.—J. J. Taubenhaus, Texas Agricultural Experiment Station, reported that root rot has now been found in 169 counties of Texas and that it has been found for the first time in Arkansas. The estimated annual aggregate loss caused by the disease in Texas, considering all crops, is approximately $100,000,000. Of this, over $60,000,000 is estimated as due to the 15 per cent average reduction in yield of cotton.

New hosts.—S. E. Wolff, Texas Agricultural Experiment Station, reported that *Allionia lanceolata* and *Psoralea tenuiflora* were found to be highly susceptible. One plant of *Salvia greggii* succumbed to root rot, the first instance reported of a mint dying from this disease. Taubenhaus summarized tests at College Station in which pomegranate, live oak, and one species of hackberry were found resistant; while yaupon and another species of hackberry were susceptible. W. J. Bach, Texas Agricultural Experiment Station, reported the following new hosts from Weslaco: anise, endive, escarole, jacaranda tree (*Jacaranda mimosaefolia*), *Schinus terebinthifolius,* and *Zelkova sinica.* The Turks-cap hibiscus (*Malvaviscus conzattii* (*arboreus*)) appeared resistant in spite of repeated inoculation. This ornamental plant is the only member of the Malvaceae known to be resistant to root rot.

Soil relations.—W. T. Carter, Soil Survey, discussed the distribution of root rot in Texas as related to soil types. Paul R. Dawson, Bureau of Chemistry and Soils, reported further on field observations in which small root-rot areas, occurring in predominantly noninfested fields of Wilson

and Irving clay soil, were closely associated with localized spots or streaks of shallow soil. These streaks were alkaline, pH 7.4 to 8.3, contained numerous concretions of calcium carbonate, and were very low in organic matter; while the noninfested areas were predominantly acid, pH 5.8 to 6.25, free of calcium carbonate, and appreciably higher in organic matter.

MORPHOLOGY AND PHYSIOLOGY OF THE FUNGUS

Taubenhaus reported studies of spore mats in which direct morphologic connections were found between the hyphae bearing the Phymatotrichum conidia aboveground and the typical Ozonium strands on roots below ground.

D. C. Neal, Bureau of Plant Industry, found that in soil cultures containing dextrose, lactose, and corn meal, considerable growth obtained but no sclerotia formed. Such competitive organisms as Aspergillus, Penicillium and *Fusarium* spp. partially arrested the development of strands of *P. omnivorum* in agar plates inoculated simultaneously. W. N. Ezekiel, Texas Agricultural Experiment Station, reported artificial culture of *Phymatotrichum omnivorum* on purely synthetic media and the production of sclerotia in such cultures.

Temperature, moisture relations.—Neal summarized experiments conducted in cooperation with W. W. Gilbert on the temperature relations of *P. omnivorum*. The minimum, optimum, and maximum temperatures for sclerotia formation appear to be 18°, 29°, and 36° C., respectively. B. F. Dana, Texas Agricultural Experiment Station, reported that soil temperatures at Temple, Texas, favor root rot from May to October, and that during this period the prevalence of the disease varies with soil-moisture conditions. Neal found that sclerotia developed in cultures in Wilson clay soil maintained at 20 per cent, 30 per cent, and 40 per cent soil moisture on an oven-dry-weight basis and that the optimum was between 35 per cent and 40 per cent.

Ezekiel reported an experiment planned to explain absence of root rot on bottom lands. Roots cut from freshly wilted cotton plants were stored in large crocks from which some were removed periodically to test the virulence of the Phymatotrichum strands on the roots by inoculation of normal cotton plants. In flooded soil, at room temperature, the fungus was virulent after three days but not after a week; in moist air, at room temperature, for two but not three weeks; and in moist air in the refrigerator, for three but not four weeks. C. J. King, Bureau of Plant Industry, found that 20 per cent of individual sclerotia of *P. omnivorum* were still viable after being immersed in water for 121 days.

LIFE HISTORY

Summer spread.—H. C. McNamara and D. R. Hooton, Bureau of Plant Industry, reported that root rot had spread in the fields of the U. S. Cotton Breeding Station at Greenville, Texas, an average of about 10 feet a year. Maps of the distribution of root rot since 1920 showed that the spots passed through cycles of increase culminating in almost complete kill, followed by abrupt decreases, often in a single year, to a few scattered areas of infection.

Winter spread, overwintering.—Taubenhaus presented studies to show that root rot spreads, not only in summer, but also on the roots of plants during the fall and winter months. Numerous experiments indicate that the fungus overwinters on infected, live roots of cotton and other susceptible plants rather than on decayed roots.

Sclerotia.—King, who first observed the production of sclerotia in cultures in the laboratory, and Neal reported separate results in which sclerotia were still viable in cultures a year old. The production of sclerotia in the laboratory in soil-filled containers was discussed by several workers. Sclerotia were first found in the field by Neal in May, 1929, and were later found by Taubenhaus and Dana. Many primary infections during 1929 were traced to the sclerotia, particularly in fields that had been in clean fallow and in grain rotation during previous years. Neal reported that 132 sclerotia, of which 90 per cent were still viable, were collected at a depth of 18 inches beneath a single cotton plant, a primary infection center in a field at San Antonio. Cultures were readily obtained from sclerotia collected in the field. Successful inoculations of cotton plants with sclerotia were reported by King, Neal, and Taubenhaus. King and Neal obtained good infection with cultures of *P. omnivorum* which had been grown on dead plant tissues and other nutrient media 11 and 12 months, respectively, prior to the inoculation; and Neal and Taubenhaus obtained infection with cultures isolated originally from sclerotia collected in the field. Taubenhaus found that the same sclerotia could grow as often as five times after the previous growth had been removed. King reported the development of a second crop of sclerotia in 4 to 5 months by budding from old clusters in cultures of sand and cotton roots that had been buried in the soil during the winter months and in similar cultures stored in the laboratory at room temperature. Small, individual sclerotia, air-dried at room temperature, were no longer viable after about 1¼ hours. Neal found similarly that single sclerotia, dried on filter paper for 6, 12, and 24 hours, respectively, at room temperature, were no longer viable. Conglomerate masses of sclerotia retained their viability after air drying for 20 hours in a calcium chloride desiccator but were killed after three days. Large hyphae in conidial mats, collected originally in an alfalfa field, retained their viability after air drying in the laboratory 6½ months.

King described the growth of *P. omnivorum* in a long glass tube containing sterile moist sand and small deposits of sterile dead cotton plant tissues placed at intervals of 2½ feet. The strands grew 11 feet in 149 days. Sclerotia developed in the strands soon after the terminal growth had advanced a few centimeters beyond the first, second, and third deposits of root tissues, but none were produced during the last 3 months of growth. Live root-rot strands were commonly found on dead tree roots several years after the trees had been removed; the roots of date palms and other plants penetrating these softened tissues were infected.

CONTROL EXPERIMENTS

Soil reaction.—Continued studies at the Texas Agricultural Experiment Station on the effect of soil reaction on root rot were summarized by Ezekiel. In a number of inoculation experiments, there were higher percentages of infection, higher percentages of plants killed, and a shorter average interval between inoculation and wilting in alkaline or neutral soils than in acid soil. Root rot was not controlled by acidification of the surface layer of soil, only. G. S. Fraps, Texas Agricultural Experiment Station, mentioned the development of a method by which the amount of acid necessary to change the soil to a desired degree of acidity may be determined. H. E. Rea, of the same station, reported field tests with sulphur in which root rot was partially controlled by high rates of application; however, there was considerable acid injury to cotton.

Rotation and clean-culture experiments were summarized by H. Dunlavy and Rea, of Texas, and McNamara and Hooton, and G. T. Ratliffe and I. M. Atkins, Bureau of Plant Industry. The results of these experiments were somewhat erratic. Clean tillage or rotation with nonsusceptible crops for one year resulted in occasional control, while two and three years of similar treatment have in some cases appreciably reduced infection.

Subsoiling experiments conducted independently by a number of investigators (Dawson, Dunlavy, McNamara, Neal, and Ratliffe and Atkins) yielded promising results in 1929. In most cases there was less root rot on subsoiled land than in adjoining check plots.

Fertilizer experiments in the black-land region of central Texas were reported by Dawson and H. V. Jordan, Bureau of Chemistry and Soils. In general, significant increases in yield resulted from the application of mixtures containing the higher proportions of phosphate together with some nitrogen, although in some instances the greatest response was to the higher proportions of nitrogen in the presence of phosphate. The increases in yield were in some cases in excess of 75 per cent and counterbalanced losses due to root rot. In several experiments, phosphatic fertilizers hast-

ened maturity; the yields were increased as bolls matured before the plants were killed by root rot.

Soil disinfectants.—King found that 1 per cent Formalin solution and 2 per cent cresylic acid were effective in checking the spread of the fungus in small isolated spots in alfalfa fields, but, in a few cases, infection reappeared in later years. The incidence and extent of infection were reduced in infested areas, flooded with these solutions before cotton was planted. Several small spots of root rot appeared late in the season and indicated failure of the treatment to destroy all sources of infection. Dana reported unsuccessful results from repeated treatments with formaldehyde in field plots. Applications of Semesan around shrubs in root-rot areas gave partial control. Bach obtained good results with copper sulphate solutions applied around citrus, grapes, and ornamentals.

Ezekiel described a laboratory method of preliminary comparison of soil disinfectants in soil chambers. The disinfectants are mixed with moist soil, placed in jars with inoculum in the center of some jars and next to the glass walls of other jars, and the extent of growth from the inoculum is then noted.

King prevented the germination of sclerotia by moistening them 45 minutes in 1 per cent Formalin solution. Neal reported that sclerotia immersed for 25 minutes in 0.5 per cent solutions of various disinfectants were still viable; Semesan reduced the viability to 13 per cent; while immersion in a 1:1000 solution of bichloride of mercury for the same time reduced the viability to 27 per cent. After exposure of sclerotia for 50 minutes to 0.5 per cent solutions of copper sulphate and chlorophenol-nitrophenol-mercury solutions, the viability was still 60 per cent and 87 per cent, respectively.

Resistant varieties.—Rea, Dana, and Dunlavy reported that, during the last three years, 11,000 varieties and strains of cotton have been tested at the Blackland Substation for resistance to root rot, and all have been eliminated as susceptible except 41 strains which are still under test. Bach tested 104 varieties of grapes and 12 rootstocks over a period of three years. The Champenel, Mustang, and Black Spanish appear resistant; and *Vitis Champini* offers promise as a rootstock. Of the citrus tested, the Sour Orange rootstock appears highly resistant; the Cleopatra orange and *Citrus trifoliata* are very susceptible; and Rusk Citrange, Thomasville Citrangequat, and Ichan Lemon have also died from the disease.

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ATTEMPTS TO INDUCE "MIXOCHIMAERA" IN FUSARIUM MONILIFORME[1]

LEON H. LEONIAN

Brierly[2] suggests that one of the underlying causes of sectorial segregations among the colonies of bacteria and fungi may be traced to the possible phenomenon of "mixochimaera" whereby germ tubes and hyphae of different strains, varieties, and even species are supposed to fuse and give rise to mycelial threads containing cytoplasm and nuclei of distinct types. He suggests that "it is by no means impossible in view of the characteristic methods of hyphal growth and direct conidial abstriction, that new strains or types of fungi might arise. . . . " If one accepts the possibility of mixochimaera, one would be justified in assuming that a mixture and a partial or total fusion between the protoplasms of different strains or species would be capable of inducing some interesting and radical deviations from the parental types. With this idea in view, the writer undertook to demonstrate the possibility of synthesizing some new strains of *Fusarium moniliforme* by artificially associating distinctly different strains in petri-dish cultures.

ORGANISMS AND TECHNIQUE

A single-spore culture of a strain of *F. moniliforme* with which the writer has worked for the last two years has exhibited an extremely plastic nature by dissociating into more than twenty-five readily recognizable variants, not to mention scores of minor ones. In common with other dissociative types, some of these variants have remained fairly constant. Others fluctuate much and give rise either to complex colonies of newer variants or revert to the older types. Variant XII, being a stable form and having shown no marked deviation from type during the course of a very intensive cultural study, was selected as one of the experimental organisms. When grown on a nutrient agar consisting of 5 grams of peptone, 20 grams of dextrose, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 20 grams of agar agar, and 1,000 cc. of distilled water, this organism forms a bright purple colony with scanty or no aerial hyphae. The second experimental organism was selected not from the foregoing variants but from another group of variants, the original of which was isolated from a totally different source. When grown on the same nutrient

[1] Approved by the director of West Virginia Agricultural Experiment Station as Scientific Paper No. 87.

[2] Brierly, William B. Variation in fungi and bacteria, Proc. Int. Cong. Pl. Sci. 1926, **2**: 1629–1654. 1929.

agar, this strain forms an orange-color colony with rather short but abundant aerial hyphae of an orange hue. During two years, when this organism was kept under constant observation, it demonstrated a remarkable constancy of behavior. The purple organism has been termed type A and the orange form type B.

All cultural work was done with petri dishes, as test-tube cultures are highly unsatisfactory and often misleading in a study of sectorial behavior. All plates were 15 mm. deep and 100 mm. in diameter and contained 15 cc. of the nutrient agar described above. Hundreds of such plates were prepared and inoculated with types A and B. In some, the hyphae of the two types were planted together; in others, spore suspensions of the two were deposited in the center of the plate. In still others, agar cultures of the two types were ground together into a paste, then a portion of the paste was planted in the dish.

RESULTS

In nearly every case a complex colony consisting of mixtures of types A and B was the result. The two types were differentiated either at the very beginning of the young colony, where the purple and the orange growth sharply separated into sectors, or an apparently pure colony of either A or B type developed. However, after assuming a diameter of 30–40 millimeters it gave rise to a sector of the other type. Just how a purple mycelium was masked by an orange colony or an orange mycelium masked by a purple one and just how it broke loose to form a sector consisting of a pure culture of only one type are not clear.

Mixed plantings often showed another behavior: type A, of slower growth habit, was sometimes left behind by the orange form, thus appearing as a purple blotch in the center of the plate. Transfers made from the outermost edge of the orange colony always gave rise to pure orange colonies. In no case did the masking effect of one or the other type extend beyond the young colony. By the time the outermost growth reached the edges of the culture dish, it was found to consist of either one or the other type only; it seems that the mixed growth cannot continue indefinitely. Sooner or later the two organisms must separate.

Hundreds of cultures invariably showed this to be the case. Even when some of the most outstanding of the 25 variants were associated together in various combinations, again there was a sharp sectorial differentiation despite the fact that all of these 25 originally came from the same single spore. The same thing was found to be true when type B was associated with other species of Fusarium, such as *F. culmorum*, *F. herbarum gibberalloides*, etc.

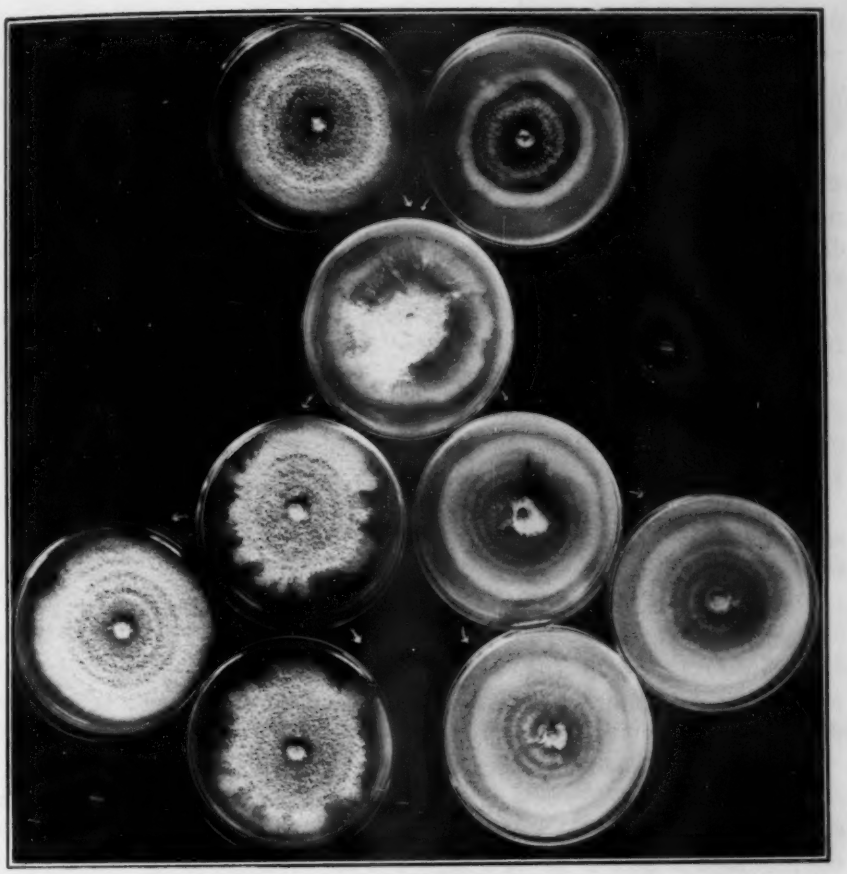

FIG. 1. Association of type A, upper right, and type B, upper left, with the resulting changes for three generations, as indicated by arrows. In the first-generation culture, second row, type A has been completely masked and suppressed by the more rapidly growing colony of type B.

In hundreds of cultures in which types A and B were associated, a new type of growth was observed at four different times. This was immediately isolated. This new type, described here as type C, resembled neither type A nor B but seemed to be an intermediate between the two. The young colonies were orange in color, thus resembling type B, but possessed no aerial mycelium, a characteristic of type A. As the colony grew older, it changed into a burnt orange, then purplish orange, and then nearly pure purple, not easily distinguished from type A. Upon growing still older, an orange-color aerial mycelium, exactly like that of type B, began to form all over the surface of the colony. Transfers made from this orange

mycelium, however, gave rise to type C colonies, only. The submerged mycelium of type C was found to be tenacious, yielding to the needle with difficulty; this holds true in case of type A, whereas type B has no tenacity and yields to the needle with great ease.

Young colonies of type A form macroconidia in great abundance and microconidia in comparatively scant numbers. Type B forms microconidia in great abundance and very few macroconidia after the colony is quite old. Very young colonies of type C form an abundance of microconidia and a few macroconidia, but, as they grow older, macroconidia appear in great numbers. One hundred single-spore isolations were made from this type, and in every case a pure colony of type C was formed. Circumstantial evidence thus pointed to a possible fusion between types A and B, giving rise to an intermediate form, type C. The only alternative was a possible dissociation of either type A or B and the formation of the new type.

While the remarkably intermediate habit of the new type at first seemed to exclude such a possibility, yet the writer's faith in a possible mixochimaera began to wane when the behavior of these strains on a different nutrient medium was observed. This medium consisted of the following ingredients: Dry malt extract 10 grams, dextrose 10 grams, potassium nitrate 2 grams, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 20 grams of agar agar, and 1,000 cc. of distilled water.

On this agar type A was almost colorless, showing only very faint zones of lavender. Type B was orange, as usual, but type C was a deep blue green. Whence came this color? A mixture of orange and purple does not give such a shade. Since a mere physical mixture of the protoplasms, of nuclei or of fractions of nuclei of types A and B could not yield this blue green shade there could be only two explanations for such a behavior: First, that either type A or B dissociated to give rise to type C and, second, that there was an actual fusion and recombination of nuclear materials of A and B. Such an explanation could rest only upon the bold and unsupported hypothesis that a sort of amorphous sexuality was operative in these fungi.

Although the appearance of this blue green pigment rendered the possibility of mixochimaera rather remote, it was deemed advisable, nevertheless, to attack the problem with the hypothesis of mixochimaera as a basis. It was reasoned that if type C represented a more or less mechanical mixture of types A and B, a differential growth factor would serve to suppress or eliminate one of the two types and allow one of them to grow. Such oxidizing or toxic agents as potassium permanganate, potassium dichromate,

silver nitrate, mercuric chloride, and Semesan were employed to split the possible mixochimaeral union. The three types of organisms were grown in a nutrient solution consisting of 10 grams each of dry malt extract and dextrose, 1 gram each of dihydrogen potassium phosphate and magnesium sulphate, 2 grams of potassium nitrate, and 1,000 cc. of distilled water. Five cc. of this solution was poured in each test tube, sterilized, and inoculated.

After seven days' incubation at 25° C. a good mycelial mat was formed on the surface of the solution. These mats were removed and washed in three changes of sterile distilled water and transferred to a series of solutions consisting of 0.5 per cent of potassium permanganate, 0.075 per cent of Semesan, and 0.02 per cent each of silver nitrate, potassium dichromate, and bichloride of mercury. An hour later they were transferred to another series of corresponding solutions with 5 cc. of the solution in each glass capsule of 25 cc. capacity. Such a double transfer served to reduce the amount of distilled water held by the mycelial mat to a negligible quantity. These cultures were set aside and examined from day to day.

Rather unexpected results followed: The potassium permanganate solution was reduced by all three types within less than a day, and new hyphae appeared and grew into the solution. Growth took place in all other solutions as well, new hyphae not only appearing over the surface of the mycelial mat but growing out into the solution of pure bichloride of mercury and similar toxic substances of relatively high concentration. The work was repeated a number of times with similar results, except that type A sometimes failed to grow even after being transferred back to a fresh supply of the nutrient solution. However, since this behavior was not constant, no reliance could be placed on the effect of differential growth on the splitting of the hypothetical mixochimaera.

In order to see if such drastic treatment exerted any effect on the characteristics of the three types, transfers of the new growths were made from these toxic solutions to plates of nutrient agar. When colonies began to form, type A retained its characteristic in all of the cultures, while some of the cultures of type B split into a complex colony consisting of types B and C, and type C also formed a complex colony consisting of types B and C. These results eliminated any possibility of mixochimaeral phenomenon and conclusively demonstrated that type C was a dissociant of type B.

At this stage of the work it seemed that the effect of oxidizing agents or toxic substances enhanced dissociation. However, the writer always has maintained that, aside from the so-called unfavorable environmental conditions and stimuli of various types, other factors also may be responsible for the appearance of dissociative phases in the cyclogeny of fungi, such as

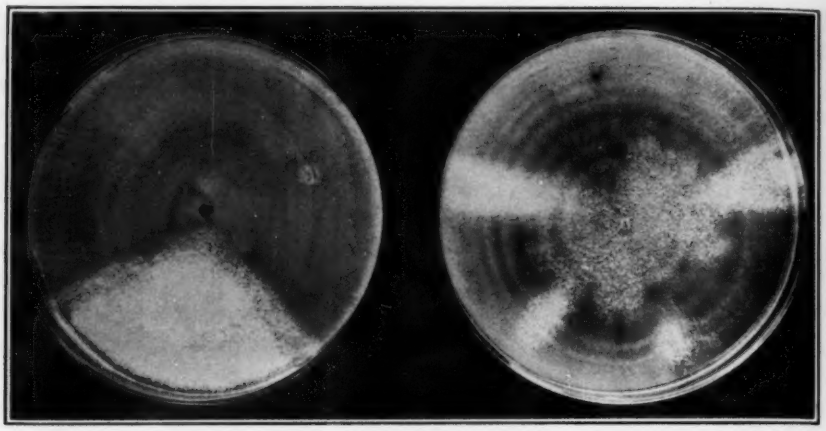

FIG. 2. Type C dissociating into type B (left); type B dissociating into type C (right).

the masking effect of different food substances, periodicity in the life cycle of organisms, and also chance and the personal-equation factors.

If one is doing an intensive cultural work with a given organism and is persistently watching for sectorial differentiations in growing colonies, one is more apt to detect and isolate dissociative phases. Because the writer assumed, to begin with, that type C resulted from the association of types A and B, his attention was centered upon an intensive study of the association of these two types. Had he made as many cultures of type B alone, he would have obtained type C as frequently and maybe more so.

In order to demonstrate this, a large number of transfers were made from the original culture of type B not only to the standard agar used in this work but to a number of others as well. Within a week a number of complex colonies consisting of types B and C resulted on all of the agars. This is by no means an unusual behavior, as similar instances of periodicity have been observed by the writer both in Fusarium and Phytophthora species. While it is not possible sharply or even approximately to delimit the chance factor from the periodicity factor, it is safe to assume that both are present and active. Intensive cultural work extending over a long period of time is necessary for the proper unraveling of all possible dissociative phases of a given organism.

CONCLUSIONS

It is concluded that, in so far as the organisms and the conditions described in this paper are concerned, mixochimaera is not a factor in dissociation phenomena. A mere mixture of protoplasms cannot give rise to new characteristics, unless there be a sexual phenomenon involved, be-

cause each nucleus will behave as a unit and will develop its own characteristics. Wherever there is a sexual attraction and fusion, sexually formed reproductive bodies result and differentiate this type of behavior from dissociation phenomena. If one were to subscribe to the possibility of the occurrence of mixochimaera, one would then be prepared to witness all sorts of bizarre combinations. An intensive study of dissociants reveals the fact that, despite all deviations from the "normal" orbit of a given species, all of its dissociants retain a remarkable continuity of kinship. Their departures are all normal variations, appearing as "abnormal" or "new" to us because of our ignorance concerning the potentialities of the species. Hyphal fusions may occur between different strains, species, and even genera, but, if anything like mixochimaera could result and if more intimate fusions could follow, taxonomy would be infinitely more chaotic than it is to-day.

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TO WHAT EXTENT IS "SPRAY BURN" OF APPLE FRUIT CAUSED BY THE FREEZING OF THE FLOWERS?

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On May 14, 1928, in the Cornell University orchard at Ithaca, New York, the temperature fell to 24 degrees F. for a short time during the night. On this date most apple varieties were in the early stages of bloom with about half of the blossoms open. In examining the closed flowers to see if they had been seriously damaged, the petals were pulled off to expose the pistils. In many cases in trying to remove the petals the outer layer or layers of cells on the entire ovary and upper part of the pedicel slipped off, adhering to the perianth. A considerable number of varieties were examined and this condition was found in practically all. The styles of many of the flowers were reddish brown but the stigmas and the young ovules were apparently uninjured. It seemed as if sufficient injury had taken place almost to eliminate the crop. Contrary to expectations, however, good crops were harvested with most varieties.

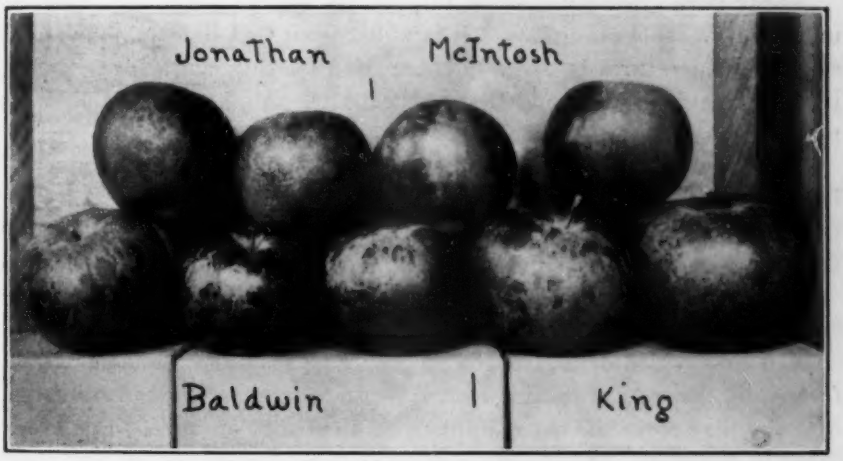

Fig. 1. Russeting of apples apparently caused by low temperature in the Cornell University orchard at Ithaca, N. Y., 1928.

During the summer and fall of 1928, it was observed that the fruit of nearly all varieties showed conspicuous russeting. The fruits shown in figure 1 illustrate the nature of this injury on the varieties Baldwin, Tompkins King, McIntosh, and Jonathan. During this season, russeting of a similar appearance was general throughout New York, particularly in

the Hudson Valley and in New England, it being much severer than usual. In most instances, growers attributed the injury to "spray burn." Although this may have been a contributing cause, it could not have been the only cause, for the injury was observed in orchards that had not been sprayed throughout the season. Further, nearly all varieties showed the injury this season. This was very unusual, as russeting from sprays is ordinarily confined to susceptible varieties, such as Baldwin and Ben Davis. It would, therefore, seem logical to suppose that the injury had been caused by the unusual cold of May 14.

In the spring of 1929 the temperature in the Cornell University orchard dropped to 29° F. on May 10 before any blossoms were open. On May 17, during the early stages of bloom, it fell to 31° F. Although some blossoms showed a little slipping of the epidermis, the injury was not frequent. During the season some netted russeting appeared in this orchard on Winter Banana and Baldwin, but not on other varieties. This lack of injury from freezing was to be expected, as 29° F. is known not to be a critical killing temperature for apple tissues. The 1929 observations thus tend to confirm the idea that the injury in 1928 was due to the low temperature prevalent while the trees were in blossom.

Spray burn often comes about through the application of spray material under unfavorable conditions. It is well known that lime sulphur applied at high temperatures is likely to cause the russeting characteristic of this injury (Heald,[1] p. 218) and that Bordeaux mixture applied at lower temperatures may have the same result (Heald,[1] p. 211).

Under some conditions, however, russeting is known to be produced by late spring freezes or frosts occurring after petal fall, entirely apart from the common forms of spray injury (Heald,[1] p. 151). Most growers are familiar with the frost bands or rings in which a band of russet extends around the fruit either at the calyx end or sometimes midway between calyx and base. This frost ring may be bulged out by the hypertrophy of the cells in that region or, again, the ring may be somewhat sunken. Not infrequently, however, russeting caused by freezing injury is much more diffuse and may cover the entire apple with a network of russet lines, as indicated in figure 1. In the season of 1928 this type of injury was clearly associated with freezing before the flowers opened. Such injury is difficult or impossible to distinguish from the real spray burn and this has led to a misunderstanding of the spray burn problem, in general.

It is entirely conceivable that russeting due to freezing may be caused by cold occurring even earlier than that reported in 1928 or shortly after the buds begin to swell. It is of interest also in this connection that hardy

[1] Heald, F. D. Manual of Plant Diseases. McGraw-Hill, New York. 1926.

varieties, such as Northern Spy and McIntosh, are less susceptible to russeting at Ithaca than are the recognizedly more tender sorts, such as Baldwin and Ben Davis.

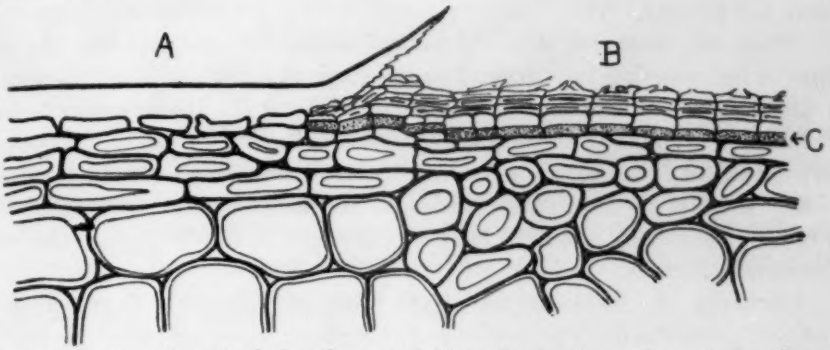

Fig. 2. Section of apple fruit taken at right angles to the surface at the edge of the russet area.

The histological nature of this injury is shown in figure 2, which represents a section through the edge of the russeted area on a fruit of Tompkins County King. At A is shown the normal cuticle and epidermis with normal fruit structure underneath. At B the normal epidermis has been destroyed and the surface covered with cork formed by the cork cambium C. It is probable that the lesion is due to the lethal freezing of epidermal cells or those near the epidermis and that the wound has been corked over by the activity of a periderm layer formed in living cells beneath the injured tissue. It will be noted that the fruit cells underneath the periderm are somewhat abnormal and apparently have arisen from the proliferation of the parenchyma beneath the injury.

The question as to why the injury occurred in a rather localized manner in some fruits and diffusely on others is difficult to explain. With a diffuse or net-like lesion it is probable that there were slight differences in the killing temperatures of the cells of the epidermis or other tissues affected and that the temperature dropped to a critical point where some cells survived and others did not. Where the russeting is localized in a continuous patch, it may be that the cells in that area were frozen to death because exposure to wind actually cooled them to a point lower than that of the uninjured parts of the epidermis or other uninjured and adjacent tissues. There is little evidence that sunlight on the frozen tissue would aggravate the injury either by more rapid thawing or by desiccation (Chandler).[2]

[2] Chandler, W. H. Fruit Growing, Chapter 21. Houghton Mifflin, New York. 1925.

It is not improbable that russeting from frost injury is aggravated or increased by the application of spray materials, which penetrate and injure the already damaged tissues more than would be possible if the frost injury were not present. This, however, needs further confirmation.

There are observations in the Cornell orchard to indicate that the nature of the russeting may depend on the stage of development of the young fruit or flowers at the time of the freeze. Where the freeze occurs before the flowers of the blossoming clusters separate, the injury has been observed to be confined to the definite sectors of the fruit, touching each other at that time. Contact of the young ovaries of the blossoms with each other or adjacent leaves may also have something to do with the frost rings that are frequently formed.

Certainly, the whole matter of the relation of freezing to russeting is not well understood. The purpose of this paper is merely to put on record observations that may corroborate the experience of others and lead to a better understanding of the whole problem, particularly as it concerns russeting caused by freezing at the time of blossoming or before, rather than after, the petals fall.

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WHEAT TAKE-ALL SYMPTOMS COMPARED WITH INJURIES CAUSED BY CHINCH BUGS[1]

HURLEY FELLOWS[2]

INTRODUCTION

In that portion of the United States where both take-all (*Ophiobolus graminis* Sacc.) and chinch bugs (*Blissus leucopterus*) are present, there is doubt sometimes as to which has caused the evident injury in some wheat fields. Both pathologists and entomologists have experienced difficulty in distinguishing between these maladies. It is much more difficult to tell the

FIG. 1. An experimental plot where most of the wheat plants had been killed by take-all and chinch bugs. In such cases detailed examinations of individual plants are necessary for proper diagnoses.

[1] Contribution No. 296 from the Department of Botany and Plant Pathology, Kansas State Agricultural College, in cooperation with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Associate Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

difference when both take-all and chinch bugs (Fig. 1) are present in the same wheat field. The present paper attempts to make clear the similarities and differences.

METHOD

Chinch bugs were caged with wheat plants growing in pots containing soil known to be free from the fungus causing take-all. The chinch bugs were allowed to feed until the plants were killed. This made it possible to observe the chinch-bug damage at all stages. Wheat plants were grown also in pots containing soil infected with *Ophiobolus graminis*.

In the field the chinch bugs were induced to congregate in the immediate vicinity of a wheat plot by spreading thin layers of straw for protection. In the spring, after the chinch bugs started migrating, cold periods made them seek the straw in large numbers. When warm weather came, they were present in multitudes to feed on the wheat plants.

COMPARISON OF SYMPTOMS

The similarity between the symptoms of the injuries caused by the two parasites is mostly in the general behavior of the wheat plants attacked. Wilting, browning, and final death of the leaves occur in both cases. This no doubt is due to the somewhat similar action of the parasites. The writer has shown that *Ophiobolus graminis* disintegrates both the phloem and the conjunctive tissue[3] of the wheat plant. Painter has pointed out that in corn, milo, and sorghum the chinch bug feeds chiefly on the contents of the phloem. He has shown also that the phloem often is plugged with special deposits of plant materials[4] and that these form a sheath around the wounds produced by the stylets of the chinch bugs. However, chinch bugs feed chiefly on the phloem in the leaf sheaths; whereas, *O. graminis* invades primary and secondary roots, subcoronal internode, crown, leaf sheath, and culm tissues. In the case of take-all, the occurrence of the fungus on other tissues as well as on the leaf sheaths is good proof of the cause of the injury.

Both take-all and chinch-bug injuries often occur in spots in the field. Chinch bugs are gregarious and apparently prefer to feed in more or less open spots where stands are thin. Take-all spots, however, may and often do occur in the interior portion of fields with very heavy stands. Furthermore, take-all spots have a very distinct margin: that is, there is a sudden transition from the short diseased plants in the spot to the surrounding healthy plants of normal height. Chinch bugs cause a more gradual transition at the margin of spots.

[3] Fellows, Hurley. Some chemical and morphological phenomena attending infection of the wheat plant by *Ophiobolus graminis*. Jour. Agr. Res. 37: 647–661. 1928.

[4] Painter, R. H. Notes on the injury to plant cells by chinch bug feeding. Ann. of Ent. Soc. America 21: 232–242. 1928.

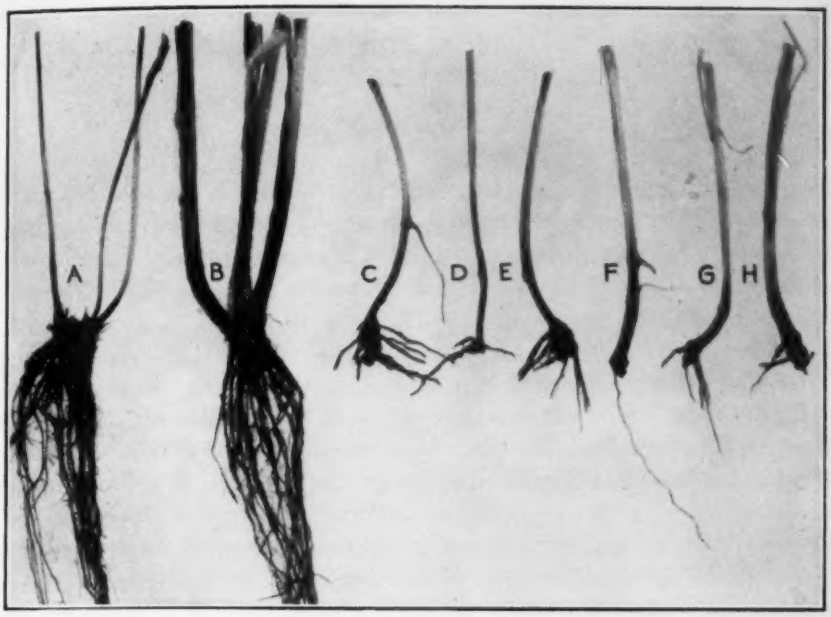

FIG. 2. Bases of wheat culms. A and B, killed by chinch bugs; C–H, killed by take-all. A, C, D, and E have the lower leaf sheaths removed, whereas the others have not.

For a reliable determination of the agent responsible for the injury, entire individual plants should be examined. Wheat plants attacked by chinch bugs have a good root system free of lesions. They also tiller well. If any discoloration occurs at the bases of the culms, it is of a brownish tinge; but it is only in the advanced stages of injury that any discolorations appear. On the other hand, the take-all fungus invades the crown, hinders the original formation of roots, and causes those that are formed to become black and brittle. The bases of the culms become black and shining. Then, too, there often is a mycelial plate on the surface of the lower culm, never present on plants injured only by chinch bugs (Fig. 2). It also has been shown by various authors that tillering is materially reduced following invasion by *Ophiobolus graminis*.

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A WASHING DEVICE FOR ISOLATION WORK WITH PLANT MATERIAL

P. M. SIMMONDS

An efficient device for washing pieces of plant tissue and seeds from which isolations are to be made has been devised and used, with quite satisfactory results, by the Dominion Plant Pathological Laboratory, Saskatoon, for the past two years. The isolations were chiefly for fungi. It has proved especially useful in making isolations from cereal grains. The usual practice when making such isolations has been to surface sterilize the plant fragments with solutions of bichloride of mercury, silver nitrate, or other chemical. With the device herein described, the pieces may be washed thoroughly with sterile water and plated immediately. Most workers have noticed that where poisons are used the growth from the isolation pieces is greatly retarded. Furthermore, it is just about impossible to use a poison on such delicate tissues as fine rootlets, etc., for, in such cases, the desired fungus may be killed outright. By washing with sterile water these disadvantages disappear, growth is rapid, and isolations are more readily obtained.

In the writer's studies many isolations were made from the fine rootlets of cereals. In using this device an increase in isolations of 100 per cent over the bichloride of mercury method was often obtained. Almost invariably there was an increase in fungi secured as well as more rapid growth. At times, when isolations were attempted from material that was not fresh or that was coated by much foreign matter, black and blue molds appeared. Black molds were most troublesome because of their rapid growth. In such cases this difficulty was largely overcome by promptly making subcultures. Washing a large number of pieces by means of a wash bottle or by rinsing becomes very tedious and laborious. The apparatus here described can wash a large number of pieces thoroughly and rapidly.

The general set-up of the apparatus is indicated in figure 1. A two litre flask, A, is fitted with a glass tube which passes through a cotton plug to connect at E. When disconnected the end of this tube may be protected by a cotton plug either wrapped or tied for security. A flask so fitted and filled with distilled water can be sterilized readily. Several such reserve flasks are kept on hand. B is a funnel plugged with absorbent cotton and serves as an air filter. A small, wide-mouth 100 c.c. flask, C, fitted through a rubber stopper with two aeration tubes, as shown, serves as washer, while flask D, connected with an ordinary filter pump at P, collects the water drawn through the apparatus. Flask C is held in place by means of swivel

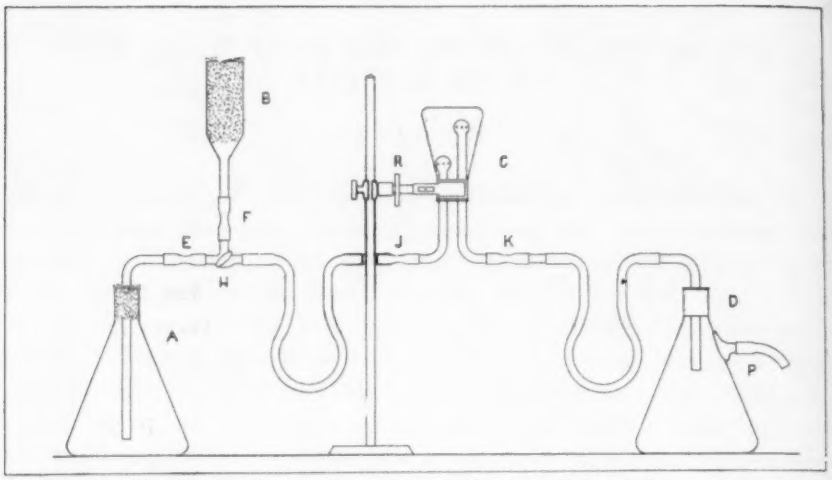

FIG. 1. A washing device for use in making isolations of fungi from plant material. A description is given in the text. (Drawn by G. Verbeke.)

clamp R and so can be inverted readily. A three-way stopcock, H, is used to control the water and air intake. Pinchcocks are used at J and K.

To use the washer, flask C is disconnected at J and K and taken from the clamp. The stopper is removed and the pieces of tissue or seeds to be washed are placed therein, after which the flask is replaced and connected. Pinchcocks at J and K are then released, the pump is started, and the stopcock at H is adjusted so as to allow water to be drawn into C. When the flask is full, H is again adjusted so as to close off the water intake but to permit air to enter through B. This will subject the material in flask C to thorough agitation. After this has continued for a minute or so and the water has decreased slightly as some is sucked over, flask C is inverted. The water is then drawn off into flask D through the aeration tube which extends to the bottom of flask C. The swivel clamp permits this movement without difficulty. After the water is drawn off the flask is returned to its former position, the air is closed off, and the water allowed to enter as before. Then the water is stopped and air introduced for agitation and so on. This rinsing or washing may be continued as many times as desirable. Experience quickly teaches when the process is complete. At the end of the process enough water is drawn into C to float or cover the material, then pinchcock K is closed, the pump is turned off, and pinchcock J is closed. This procedure prevents any back pressure. Flask C is now disconnected at J and K, using these pinchcocks and rubber tubing to close the ends of the aeration tubes. The material may then be removed with sterile forceps under ordinary aseptic conditions. By having several

washer flasks fitted up one man can be plating while another is manipulating the apparatus. Thus a large number of pieces can be plated in a relatively short time.

It should be mentioned that when the washing is in progress flask C may need to be shaken at times to remove material that may lodge against the outlet aeration tube. The holes in these tubes are not always small enough to prevent very fine material from passing through; in such cases a fine-mesh copper wire screen can be sealed into the ends of ordinary glass tubes to replace the aeration tubes. Other adaptations may be made, depending upon the circumstances.

If a three-way stopcock is not available, an ordinary Y connection may be employed by the proper manipulation of pinchcocks at E and F. The material should be washed under the tap as cleanly as possible before cutting it up into the pieces from which isolations are to be made.

DOMINION LABORATORY OF PLANT PATHOLOGY,
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SMUT RESISTANCE IN MILLET

R. H. Porter,[1] H. K. Chen, and T. F. Yu

In previous papers[2,3] the writers mentioned the seriousness of kernel smut of millet (*Ustilago crameri* (Körn)) in north China. In the summer of 1925, while inspecting some cooperative experimental plots in the provinces of Honan and Shantung, the senior writer made a number of head selections from a millet field near Wiehsien, Shantung, and at the same time he secured from Mr. E. J. Middleton, at Kaifeng, seed samples of a number of millet varieties and selections.

In the spring of 1926 an experiment was planned to test the reaction of these selections and varieties of millet to kernel smut. About 700 head selections and 57 other samples, some of which were varieties, were included in this first test. Seed of each sample was inoculated with smut spores by means of a tea strainer in which the seed and smut powder were mixed together. After the smut was strained through the sieve, the seed was placed in an envelope to be used later for planting.

The inoculated seed was measured out in approximately 3-gram samples and planted in rows 5 feet long with a foot between each row. Every tenth row was planted with inoculated seed of a common susceptible variety as a check. Unfortunately this seed used for the check was not viable, so that no readings could be made, but the percentage of smut was so high in most of the selections that there was no question about the effectiveness of the method of inoculation. Records on all of the rows were not preserved for 1926, because the smut infection ran as high as 70 per cent in some cases and these selections were discarded. The inoculum used in 1926 and in subsequent years was gathered from smutted heads in a field at Weihsien, Shantung, in 1925. All plantings were made on the experimental plots of the University of Nanking, Nanking, China.

In the succeeding years, some slight variation in the method of planting was made; for example, in 1927 and 1928, the rows were 8 feet and 16 feet long, respectively, and each plot was duplicated and planted in a different location. In 1929 only the most promising selections were included and each sample was replicated 10 times in rows 16 feet long. In addition, 19 of these selections were planted in the greenhouse in rows 1½ feet long. The

[1] At the time these investigations were initiated, the senior writer was Plant Pathologist at the University of Nanking, Nanking, China.

[2] Porter, R. H. Seed disinfectants for the control of kernel smut of foxtail millet. Proc. 3rd Pan-Pac. Sci. Cong., Tokio. Pp. 2103–2107. 1926.

[3] Porter, R. H., T. F. Yu, and H. K. Chen. The effect of seed disinfectants on smut and on yield of millet. Phytopath. **18**: 911–919. 1928.

planting dates were: June 23, 1926; June 22, 1927; June 20, 1928; and June 14, 1929. The results of the four-year test are presented in table 1, in which both resistant and susceptible strains are listed for purposes of comparison.

TABLE 1.—*Reaction of varieties and strains of millet to kernel smut caused by Ustilago crameri*

Nanking No.	Average per cent smut					Source No.
	1926	1927	1928	1929		
	Field	Field	Field	Field	Green-house	
2	0	0	0	.....	.....	Shansi 1
3	0	8.2	45.5	.....	.....	Kaifeng 3
6	Trace	0.9	1.8	1.7	4.8	" 16
9	Trace	0.9	1.2	2.7	10.0	" 20
12	0	0	0	0.3	0	" 23
13	0	0	1.4	.....	.....	" 24
16	0	0	0	7.6	0	" 27
17	0	0	0	1.1	0	" 28
18	0	0	0	0.1	0	" 29
19	0	0	0.4	0	0	" 30
20	.....	0	27.4	.....	.....	" 31a
26	0	0	0	0.1	0	" 31b
28	Trace	0	0.9	2.7	0	" 41
29	Trace	0.3	2.0	1.5	0	" 43
30	0	0	2.1	0.1	0	" 44
31	0	0	0	0.3	0	" 45
35	0	0.2	0	0.2	0	" 49
37	0	0	0	1.7	0	" 51
38	Trace	0.2	0	1.8	.....	" 52
39	0	2.7	9.7	.....	.....	" 53
42	.....	22.7	26.1	.....	.....	" 56
45	0	0	4.2	.....	.....	" 59
47	0	0	0	0	0	" 61a
48	.....	0	7.6	.....	.....	" 61b
53	.....	3.6	16.9	.....	.....	" 72
56	0	0	0	1.2	0	" 80
57	0	0	0	0	0	" 81
58	.....	17.7	50.8	.....	.....	" 85
H–28	.....	.....	1.3	0.4	0	Peking-Sel.
H–278	.....	.....	42.8	.....	.....	" "
Inoculated checks	Seed not viable	7.1	33.2	35.1	51.0	

The results in the above table show that about 20 of the millet selections are highly resistant to smut; in fact, two showed no smut in any of the tests and ten others showed only a trace in one or two years out of four. Apparently, there are strains or varieties of millet resistant to kernel smut, a fact that may prove of great value in the control of the disease.

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AMES, IOWA.

PHYTOPATHOLOGICAL NOTES

Two new diseases of cultivated mushrooms.—During the past year commercial mushroom growers have suffered considerable loss and inconvenience from two diseases that have not been described in the literature. The symptoms and etiology of these diseases are quite different and they are apparently in no way associated. The outstanding characteristic of one of them is the malformation of individual mushrooms by the production of numerous intumescences and patches of gills over the surface of the pileus. These hypertrophied growths often resemble the rose-comb of poultry and have suggested the name *rose-comb disease.* The other disease is characterized by a reduction in the yield of mushrooms, especially during the latter half of the crop, coincident with the appearance over the surface of the soil of wrinkled discoid patches of fungus tissue and, throughout the compost, of cerebriform subglobose fungus bodies. These bodies are the ascocarps of an undescribed species referred to the genus Pseudobalsamia, and they suggest the name *truffle disease.*

For several years occasional specimens typical of the *rose-comb disease* have been submitted to the Office of Mycology and Disease Survey. Dur-

FIG. 1. *Rose-comb disease* of the "snow white" variety of mushrooms, received from a commercial grower in Chester County, Pennsylvania. Photographed by J. F. Brewer.

ing the past season the disease was unusually prevalent and complaints accompanied by specimens were received from New York, Pennsylvania, Ohio, and Missouri. As mentioned above, the principal symptoms are the production of wart-like intumescences, deep seams, and superfluous gills over the surface of the pileus (see Fig. 1). They are strongly suggestive of crown gall, and bacteria occasionally may be isolated from the hypertrophied tissue. But these organisms have not caused tumors upon reinoculation and do not resemble the crown-gall organism. Furthermore, a virulent culture of *B. tumefaciens*, loaned to me by Miss Nellie A. Brown, Office of Horticultural Crops and Diseases, was innocuous when injected into healthy mushrooms. The agencies that seem most likely to be the cause of the tumors are coal-oil fumes or the fumes or oxidation products of materials contained in mineral oil. In all of the cases I have been able to investigate the disease was correlated with the use of some form of mineral oil in the mushroom houses, and the discontinuance of this practice eliminated the trouble. In several cases the disease was correlated with the use of a proprietary insect spray containing pyrethrum and kerosene, in others with the excessive use of kerosene in fly traps and as a direct spray, and in one case with the application, to mushroom beds, of water containing lubricating oil that came from an expansion tank made from a discarded oil drum. Instances of this kind are clear-cut and apparently too numerous to be mere coincidences. On the other hand, it must be admitted that we have not yet determined the factors necessary for the consistent production of the symptoms under experimental conditions.

The *truffle disease* was first called to my attention in April, 1929, by a grower at Ashtabula, Ohio. Since then I have observed it in Minnesota, Pennsylvania, and New York and have received typical specimens from Colorado. Under certain conditions the disease may completely prevent the development of the latter half of the crop in an infested house. It is especially serious in slow-growing "runs." During the past season in New York, one grower alone suffered a loss from the disease of at least $100,000. After the first few "breaks" affected beds can be easily recognized by the development of fungus wefts and rudimentary mushrooms under the sideboards and sometimes over the casing soil and by the growth over the soil and in the compost of yellowish wrinkled fruiting bodies that are discoid on the surface and subglobose in the compost. The disks range in size from that of a dime to a half-dollar, while the subsurface fruiting bodies are from 3 to 30 millimeters in diameter. They are the ascocarps of an undescribed species of truffle which undoubtedly is the cause of the diseased condition of the beds. The species (to be described in a current number of Mycologia) is apparently most closely related to the genus Pseudobalsamia, but

it has smaller spores and differs in other respects from any known truffle. The ascocarps usually begin to appear scattered over the surface of the soil and in the compost about a week after the area inhabited by the fungus has ceased bearing mushrooms.

Very little is known regarding the source of the truffle, the factors affecting its accumulation in the compost, its relation to the cultivated mushroom, its persistence from one crop to another, and control measures. Circumstantial evidence suggests that it is a widely distributed soil organism which is inconspicuous until it enters the mushroom house and finds a favorable medium for extensive development in the bed. Whether it is a parasite or acts as a weed in the bed is problematic. In some cases it has seriously affected one or two crops in a mushroom establishment and, later, for no apparent reason, failed to develop in subsequent crops.—EDMUND B. LAMBERT, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Cleaning up strawberry stock infested with root-gall nemas.—Observations for several years, in the strawberry breeding grounds at the U. S. Plant Field Station at Glenn Dale, Md., seemed to indicate that, with care, it was a comparatively easy matter to greatly improve any stock of strawberries infested with the root-knot nematode (*Caconema radicicola*). In 1928, several selections were so badly infested that they could not be distributed. These were planted on a relatively heavy soil free of root-gall nemas with the idea that runner plants would be free from the nema. In 1929, when runner plants were dug, they were, in nearly every instance, found clean. A more severe test was therefore made. Plants of a variety with the heaviest infestation yet observed (Fig. 1, A) were brought to the Station and planted on a nema-free clay loam. In the spring of 1930, the old plants showed some galls but far fewer than the set plants. The runner plants, however, were entirely clean (Fig. 1, C). The reason for the clean runner plants from the infested mother plants is the known fact that the nemas have difficulty in moving through heavy soils and were, therefore, unable to penetrate the clay soil to reach the roots of the young plants.

Strawberry-plant growers of necessity locate their nurseries on light soils because the plants can be dug in the spring when they could not be dug from heavy soils. Moreover, the root systems of plants from light soils are considered better. However, many plant growers can select small areas of relatively heavy soil to use in propagating a clean stock for their own planting. If a clay soil cannot be used, the cleanest plants from the edges of rows grown on fine sandy soil may be used to set a second bed in nema-

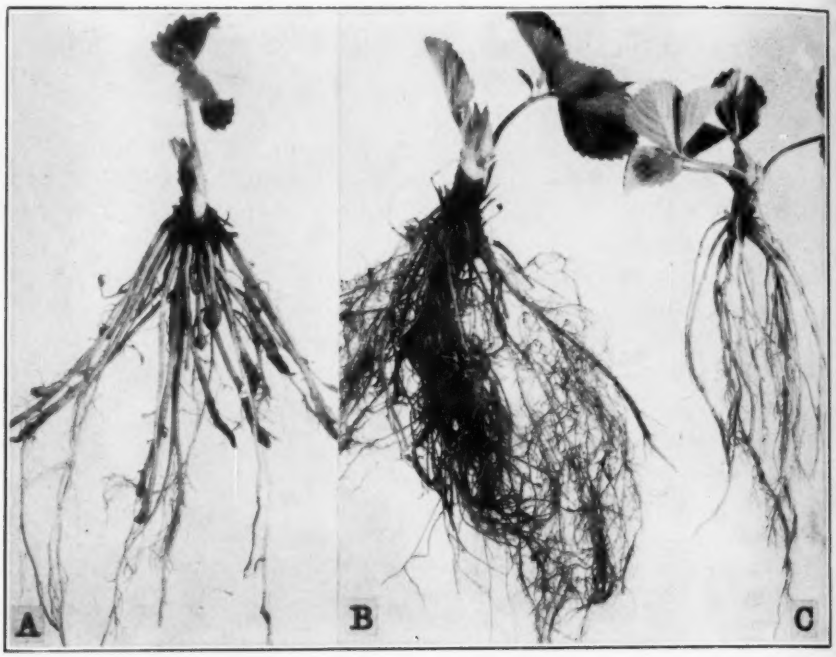

FIG. 1. The three plants shown are all of the same variety, U. S. D. A. No. 657, of strawberry. Plant A was grown at Willard, N. C., and shows the infestation there by the root-knot nematode. Plant B was a similarly infested plant but taken to Glenn Dale, Md., and grown in heavy soil for one year. A few infested roots can be seen, but the infestation is much less than when it was planted. Plant C is a runner plant grown from infested plants at Glenn Dale, Md., in heavy soil. No galls or indications of the nema were found on it. With care clean stocks can be propagated from infested stocks by growing on clay soils.

free soil and, with care, clean stocks can be obtained in two years.—GEORGE M. DARROW and GEORGE F. WALDO, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Carrot and parsley yellows transmitted by the six-spotted leafhopper, Cicadula sexnotata (Fall.).—A number of plant pathologists have called attention to a disease of carrots having most of the characteristic symptoms of yellows, but whether it was caused by the aster-yellows virus remained to be determined. In New York, Whetzel[1] reported a yellows disease of carrots ranging from a trace to 1 or 2 to 25 per cent infection in the Williamson area. Folsom[2] found apparently the same carrot disease

[1] Whetzel, H. H. Diseases of muck crops in New York. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 115–118. 1929.

[2] Folsom, D. Yellows. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 148–149. 1929.

as described by Whetzel, at Orono, Maine, and on the experimental farm in the southwestern part of the State. Zundel[3] reported the observation of yellows believed to be caused by the aster-yellows virus in carrots in Cumberland County, Pennsylvania.

During the spring and late summer of 1929 a survey was made of the yellows of plants grown on the ranch of the Morse Seed Company located in the Salinas Valley, California. It was observed during early June that the inner leaves of many varieties of carrots were yellow, but the plants were small and the symptoms were just beginning to develop. During late summer parsley and the white varieties of carrot showed some of the characteristic symptoms of yellows but, unfortunately, the varieties of yellow carrots had been pulled.

Experiments demonstrated that noninfective six-spotted leafhoppers, *Cicadula sexnotata*, after feeding on White Belgian, Short White carrots, and Hamburg parsley naturally infected with yellows, became infective and transmitted typical yellows to healthy asters and celery. Leaf hoppers, after feeding on the infected asters and celery, transmitted the disease back to the same varieties of healthy white carrots and parsley.

The same varieties of carrot and parsley grown from seeds were experimentally infected with yellows by infective leafhoppers. After symptoms of yellows developed, noninfective six-spotted leafhoppers feeding on the experimentally infected plants became infective and transmitted the disease to healthy asters and celery. The disease was transmitted from infected asters and celery back to healthy carrots and parsley. Yellows disease was also transmitted from infective carrots to healthy ones and similarly from parsley to parsley, carrot to parsley, and parsley to carrot. These experiments proved that the virus of carrot, parsley, celery, and aster yellows is identical.

Carrots and parsley infected with yellows showed a marked yellowing of the younger central leaves, while the older outer leaves were usually reddened. The younger central leaves were dwarfed and the petioles sometimes twisted; occasionally a dense growth of adventitious chlorotic shoots developed at the center of the crown. The roots were usually dwarfed, with bunched rootlets.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, and United States Department of Agriculture, Bureau of Entomology, Berkeley, California.

[3] Zundel, G. L. Yellows (virus) on various plants. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 13: 174. 1929.

Pear blight on Cotoneaster.—Species of Cotoneaster in the neighborhood of Riverside, California, are frequently infected with the pear-blight organism (*Bacillus amylovorus* (Burrill)). During the spring of 1929 a definite pathogene was isolated from a species of Cotoneaster. This was grown in pure culture and showed the characteristic growth of *B. amylovorus* in the media tested. Artificial inoculation on shoots of *Pyrus communis* (pear) and *Cotoneaster panosa* gave typical lesions. The organism was subsequently reisolated from these inoculations. So far as the writer knows, Cotoneaster has never before been tested as a host for *B. amylovorus*.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

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